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COMMON BEAN (*Phaseolus vulgaris* L.) YIELD, ROOT GROWTH, AND N  
FIXATION RESPONSE TO MOISTURE DEFICITS

by

Maurice D. Yabba

A DISSERTATION

Submitted to  
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## ABSTRACT

### COMMON BEAN (*Phaseolus vulgaris* L.) YIELD, ROOT GROWTH, AND N FIXATION RESPONSE TO MOISTURE DEFICITS

by

Maurice D. Yabba

Common bean (*Phaseolus vulgaris* L.) is grown on more than 12 million hectares and constitutes the most important food legume for more than 500 million people in Latin America, the Caribbean, and Africa, where it is often grown under moisture deficits in soils with non-optimal pH. The objectives of this study utilized limiting and non-limiting moisture regimes to determine (i) if selected genotypes of common bean exhibited differences in drought resistance as measured by yield, (ii) if drought resistant genotypes had differing root growth, and (iii) if genotypes differed for N fixation. Field studies were conducted at the Agricultural Experiment Station in St. Croix, USVI in 1999 and 2000 to evaluate the effect of moisture deficits on seed yield. Yield of the nine genotypes ranged from 142 to 1508 kg ha<sup>-1</sup> in 1999 and 568 to 896 kg ha<sup>-1</sup> in 2000. In both years, yield was affected by infestations of common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*), *Cercospora* (*Cercospora canescens*), and N-deficiency. Geometric mean ranked PR9603-22 and the nodulated (nod) and non-nodulated (nn) isolines of DOR 364, among the top four genotypes with regard to drought resistance in 1999 and 2000. Root length was quantified for 10 root width classes with diameters ranging from 0.01 - 4.5 mm. Plants in growth pouches (25.4 x 35.6 cm) were grown in the growth chamber

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containing half-strength Hoagland's nutrient solution (control) or half-strength Hoagland's nutrient solution +  $10^{-6}$  M (abscisic acid) ABA. The ABA treatment significantly increased total root length (TRL), root length of various root width classes, and root and shoot dry weight. Generally, XAN 176 and SEA5 had a higher TRL than the other genotypes and both had the highest root and shoot dry weight. For plants grown in polyvinyl chloride tubes [(PVC) 0.35 x 0.92 m], water deficit significantly reduced root length in root width classes at all depths except 30.6 - 45.7 cm and reduced TRL by approximately 75, 38, and 38% at depths of 0 - 15, 15.1 - 30.5, and 0 - 92 cm, respectively. The genotypes XAN 176 and SEA5 were consistently among the lines producing the greatest root length in both stress and non-stress environments.

Approximately 97 and 93% of all roots were in root classes  $\leq 1$  mm in diameter in plants grown in growth pouches and PVC tubes, respectively. N fixation was estimated via the N difference method, using non-nodulating (nn) isolines of BAT 477 and DOR 364 as the reference crops. Total N-fixed among the genotypes was low, ranging from no fixation (-34.3 kg ha<sup>-1</sup>) to 19.9 kg ha<sup>-1</sup>. DOR 364 (nn) gave a higher estimate of N-fixation than did BAT 477 (nn). BAT 477 (nodulated) was one of the genotypes with the highest root-N concentrations as were the higher yielding genotypes XAN 176 and PR9603-22.

Nitrogen harvest index values among genotypes ranged from 7 to 76%. Nitrogen use efficiency did not differ among irrigated and rainfed treatments in 1999 but was greater in the irrigated treatment in 2000. Genotypes varied for yield, TRL, NUE, NHI, and N fixation. Growth pouch and PVC studies identified XAN 176 and SEA5 as having high TRL, suggesting that growth pouches may be a viable method for assessing root growth of differing lines.

In loving

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**Sundra Philetta Yabba**

**for the joy and happiness you brought into my life**

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## TABLES OF CONTENTS

LIST OF TABLES.....	x
LIST OF FIGURES.....	xvii
LITERATURE REVIEW.....	1
Introduction .....	1
Diseases.....	2
Drought.....	4
Roots.....	6
Nitrogen Fixation and its effect on drought resistance.....	8
Literature cited.....	11
CHAPTER 1	
COMMON BEAN ( <i>Phaseolus vulgaris</i> L.) YIELD UNDER HIGH pH, LIMITED MOISTURE , AND LOW NITROGEN	
Abstract.....	19
Introduction.....	20
Materials and Methods.....	21
Field study.....	21
Water regime.....	22
Plant material.....	22
Experimental design.....	23
Data collection.....	23
Moisture stress indices.....	24
Results and Discussion.....	25
1999.....	25
2000.....	30
Effect of pH on yield of common bean.....	32
Nutritional and pathological problems.....	34
Conclusion.....	35
Literature cited.....	36
CHAPTER 2	
ROOT LENGTH, SHOOT WEIGHT, AND ROOT LENGTH DENSITY IN COMMON	

BEA

CHAPT  
NITRO  
CENTR  
UNDEF

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(

BEAN ( <i>Phaseolus vulgaris</i> L.)	
Abstract.....	50
Introduction.....	51
Materials and Methods.....	53
Growth chamber study.....	53
Glasshouse study.....	54
Root quantification.....	55
Results and Discussion.....	56
Root parameters: Growth chamber study.....	56
Root parameters: Glasshouse.....	59
Shoot and root dry weight and R/S.....	66
Control genotypic response.....	66
ABA genotypic response.....	67
PVC genotypic response.....	68
Conclusion.....	71
Literature cited.....	72

### CHAPTER 3

#### NITROGEN FIXATION AND PARTITIONING OF NINE CARIBBEAN AND CENTRAL AMERICAN COMMON BEAN (*Phaseolus vulgaris* L.) LINES GROWN UNDER RAINFED AND GLASSHOUSE CONDITIONS

Abstract.....	98
Introduction.....	99
Materials and methods.....	100
Field Study.....	100
Plant material.....	101
Experimental design.....	101
Data collection.....	102
N <sub>2</sub> fixation.....	102
Glasshouse study.....	103
Statistics.....	103
Results and Discussion.....	103
Partitioning.....	103
Water effect.....	103
Root-N concentration.....	104
Stem-N concentration.....	105
Leaf-N concentration.....	106
Reproductive-N concentration.....	107
Nitrogen harvest index.....	108
Harvest index.....	110
Nitrogen use efficiency.....	111
Nitrogen fixation.....	112
Conclusion.....	114

Literature cited.....	115
Summary and conclusions.....	137
Appendix.....	139
Appendix A.....	139
Appendix B.....	143

CH:

Tab  
Agr  
Cro

Tab  
fill  
Agr  
U.S  
199

Tab  
per  
pod  
at th  
Car

Tab  
(Ce  
gro  
Cro  
dea

Tab  
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ind  
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U.S  
200

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(P)

## LIST OF TABLES

### CHAPTER 1

- Table 1. Characteristics of common bean genotypes grown in field experiments at the Agricultural Experiment Station at the University of the Virgin Islands, Christiansted, St. Croix, U.S.V.I. in 1999 and 2000.....40
- Table 2. Days to flower (DF), days to physiological maturity (DPM), and days to seed fill (DSF) of nine common bean (*Phaseolus vulgaris* L.) genotypes grown at the Agricultural Research Station at the university of the Virgin Islands-St. Croix Campus, U.S.V.I. 1999.....41
- Table 3. Yield under stress and nonstress treatments (kg ha<sup>-1</sup>), combined yield (kg ha<sup>-1</sup>), percent yield reduction, geometric mean (GM), number of pods harvested per plot, and pod weight per plot (g), of nine common bean (*Phaseolus vulgaris* L.) genotypes grown at the Agricultural Research Station at the university of the Virgin Islands-St. Croix Campus, U.S.V.I. 1999..... 42
- Table 4. Common blight (CB) (*Xanthomonas campestris* pv. Phaseoli), Cercospora (*Cercospora canescens*), and ozone rating of nine bean (*Phaseolus vulgaris* L.) genotypes grown at the Agricultural Research Station at the University of the Virgin Islands-St. Croix Campus, U.S.V.I. 1999. Scale 0 to 9 with 0 = no visual symptoms and 9 = death..... 43
- Table 5. Yield (Kg ha<sup>-1</sup>) under irrigated and rainfed conditions, combined yield (Kg ha<sup>-1</sup>), percent yield reduction, 50 seed weight, seed per pod, number of pods harvested, pod weight, geometric mean (GM), drought susceptible index (DSI), and stress tolerance index (STI) of eight common bean (*Phaseolus vulgaris* L.) genotypes grown at the Agricultural Research Station at the university of the Virgin Islands-St. Croix Campus, U.S.V.I. 2000.....44
- Table 6. Correlations of yield under stress, yield under non-stress, and combined yield for stress and non-stress treatments to geometric mean (GM), drought susceptible index (DSI), and stress tolerance index (STI). Data from eight genotypes of common bean (*Phaseolus vulgaris* L.) grown at the Agricultural Research Station at the University of



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the Virgin Islands-St. Croix Campus, U.S.V.I. 2000.....45

## CHAPTER 2

Table 1. Total root length (TRL) and root length (RL) (cm) of eight common bean genotypes germinated in a germination chamber for 4 d at 25°C and transplanted to an environmentally controlled growth chamber for 28 d at 23/20°C day/night temperatures and a 15 h photoperiod and grown under control conditions in a half-strength Hoagland's solution or in 10<sup>-6</sup> M ABA solution. Roots were harvested at 14, 21, and 28 days after transplanting (DAT) and divided into 10 classes based upon root diameter. n = 32.....78

Table 2. TRL (m) at each harvest date for eight genotypes of common bean (*Phaseolus vulgaris* L.) plants germinated in a germination chamber for 4 d at 25°C and transplanted to an environmentally controlled growth chamber for 28 d at 23/20°C day/night temperatures and a 15 h photoperiod and grown under control conditions in a half-strength Hoagland's solution or in 10<sup>-6</sup> M ABA solution. N = 4 .....79

Table 3a and b. Root length (RL) of nine different root width <sup>1</sup>classes of common bean grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions. n = 27.....80

Table 4. Total root length (m) (TRL) at 15.24 cm depth increments for nine common bean genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions. n = 6.....82

Table 5. Statistical significance from ANOVA for genotypes, water, and genotype x water interaction for all root width classes and rooting depths of nine common bean genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions. n = 6 (genotypes), 27 (water), and 3 (genotype x water)..... 83

Table 6. Statistical analysis from ANOVA for genotypic response of nine common bean genotypes for all root width classes and rooting depths when grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions. n = 3.....84

Table 7. Combined root length (cm) from stressed and nonstressed moisture conditions

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of nine common bean (*Phaseolus vulgaris* L.) genotypes of root width class 3, 4, and 10 at depth “A” and root width class 3 at depth “B” and “C” grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod in stress and non-stress conditions. n = 6.....85

Table 8. Combined root length (cm) of root width classes 1, 2, 3, 4, 5, 6, and 10 at depth “D” (45.8 - 61 cm), from stressed and nonstressed moisture conditions of nine common bean (*Phaseolus vulgaris* L.) genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod. n = 6 .....86

Table 9. Total RL (cm) of root width classes 1, 2, 3, 4, 5, and 10 at depth “B” (15.3 - 30.5 cm) for nine common bean (*Phaseolus vulgaris* L.) genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under moisture stress conditions. n = 3.....87

Table 10. Root length (cm) of nine common bean (*Phaseolus vulgaris* L.) genotypes of root width classes 1, 2, 3, 4, and 10 at depth “B” (15.3 - 30.5 cm) grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under moisture stress conditions. Means ± SE, n = 3.....88

Table 11. Root length (cm) for root width classes 1, 2, 3, 4, 5, 6, and 10 at a depth of 45.8 - 61 cm (D) for nine common bean (*Phaseolus vulgaris* L.) genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under moisture stress conditions. n = 3.....89

Table 12. Total root dry weight (RDW), root length (RL), average root diameter (RD), average root surface area (RSA), average root volume (RV), and root length density (RLD), for all root width classes of common bean plants grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed conditions. n = 27.....90

Table 13. Root length density (RLD) for nine genotypes of common bean (*Phaseolus vulgaris* L.) plants grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions at two soil depths, 15.3 to 30.5 and 45.8 to 61 cm. n = 3 (stress and nonstressed RLD) and 6 (combined RLD).....91

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Table 14. Dry weight (g) of shoot and root and root/shoot ratio of eight common bean genotypes germinated in a germination chamber for 4 d at 25°C and transplanted to an environmentally controlled growth chamber for 28 d at 23/20°C day/night temperatures and a 15 h photoperiod and grown under control conditions in half-strength Hoagland's solution or in 10<sup>-6</sup> M ABA solution. n = 32.....92

Table 15. Shoot and root dry weight (g) and root/shoot ratio of eight common bean genotypes germinated in a germination chamber for 4 d at 25°C and transplanted to an environmentally controlled growth chamber for 28 d at 23/20°C day/night temperatures and a 15 h photoperiod, grown in a half-strength Hoagland's nutrient solution, and sampled at 14, 21, and 28 DAT. n = 4. Control treatment.....93

Table 16. Shoot and root dry weight (g) and root/shoot ratio of eight common bean genotypes germinated in a germination chamber for 4 d at 25°C and transplanted to an environmentally controlled growth chamber for 28 d at 23/20°C day/night temperatures and a 15 h photoperiod, grown in a 10<sup>-6</sup> M ABA, and sampled at 14, 21, and 28 DAT. n = 4. ABA treatment.....94

Table 17. Dry weight (g) of leaves, stems, reproductive parts, shoots, and root and root/shoot ratio of nine common bean genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions. n = 27.....95

Table 18. Dry weight (g) of leaves, stems, reproductive parts, shoots, and root and root/shoot ratio of nine common bean genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions. n = 6.....96

Table 19. Dry weight (g) of leaves, stems, reproductive parts, shoots, and root and root/shoot ratio of nine common bean genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions. n = 3.....97

### CHAPTER 3

Table 1. The effect of moisture stress on root, stem, leaf, and reproductive structures-N concentration (g kg<sup>-1</sup>) in common bean (*Phaseolus vulgaris* L.) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 1999 and 2000, under irrigated and rainfed moisture conditions and in polyvinyl chloride (PVC) tubes in a greenhouse at Michigan State University, East Lansing, MI. in 2000, under stressed and

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Tab

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irrig

Mic

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(co

Tab

(Ph

gro

199

irrig

nonstressed moisture condition. N = 36 (UVI), N = 27 (PVC).....	120
Table 2. Root-N concentration (g kg <sup>-1</sup> ) of nine common bean ( <i>Phaseolus vulgaris</i> L.) genotypes harvested at three growth stages (V3, R2, and R7) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 1999 under rainfed and irrigated moisture conditions. N = 8 (combined), 4 (rainfed or irrigated).....	121
Table 3. Root-N concentration (g kg <sup>-1</sup> ) of nine common bean ( <i>Phaseolus vulgaris</i> L.) genotypes harvested at three growth stages (V3, R4, and R8) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 2000 under rainfed and irrigated moisture conditions. N = 8 (combined), 4 (rainfed or irrigated).....	122
Table 4. Stem-N concentration (g kg <sup>-1</sup> ) of nine common bean ( <i>Phaseolus vulgaris</i> L.) genotypes harvested at three growth stages (V3, R2, and R7) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 1999 under rainfed and irrigated moisture conditions. N = 8 (combined), 4 (rainfed or irrigated).....	123
Table 5. Stem-N concentration (g kg <sup>-1</sup> ) of nine common bean ( <i>Phaseolus vulgaris</i> L.) genotypes harvested at three growth stages (V3, R4, and R8) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 2000 under rainfed and irrigated moisture conditions and in polyvinyl chloride (PVC) tubes in a greenhouse at Michigan State University, East Lansing, MI. in 2000, under stressed and nonstressed moisture conditions. N = (UVI)8 (combined), 4 (rainfed or irrigated); (PVC) N = 6 (combined), 3 (stress or nonstress).....	124
Table 6. Leaf-N concentration (g kg <sup>-1</sup> ) of nine common bean ( <i>Phaseolus vulgaris</i> L.) genotypes harvested at three growth stages (V3, R2, and R7) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 1999 under rainfed and irrigated moisture conditions. N = 8 (combined), 4 (rainfed or irrigated).....	125
Table 7. Leaf-N concentration (g kg <sup>-1</sup> ) of nine common bean ( <i>Phaseolus vulgaris</i> L.) genotypes harvested at three growth stages (V3, R4, and R8) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 2000 under rainfed and irrigated moisture conditions and in polyvinyl chloride (PVC) tubes in a greenhouse at Michigan State University, East Lansing, MI. in 2000, under stressed and nonstressed moisture conditions. N = (UVI) 8 (combined), 4 (rainfed or irrigated); (PVC) N = 6 (combined), 3 (stress or nonstress).....	126
Table 8a and b. Reproductive structures-N concentration (g kg <sup>-1</sup> ) of nine common bean ( <i>Phaseolus vulgaris</i> L.) genotypes harvested at three growth stages (V3, R2, and R7) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 1999 under rainfed and irrigated moisture conditions. N = 8 (combined), 4 (rainfed or irrigated).....	127



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Table 9. Reproductive structures-N concentration (g kg <sup>-1</sup> ) of nine common bean ( <i>Phaseolus vulgaris</i> L.) genotypes harvested at three growth stages (V3, R4, and R8) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 2000 under rainfed and irrigated moisture conditions and in polyvinyl chloride (PVC) tubes in a greenhouse at Michigan State University, East Lansing, MI. in 2000, under stressed and nonstressed moisture conditions. N = (UVI) 8 (combined), 4 (rainfed or irrigated); (PVC) N = 6 (combined), 3 (stress or nonstress).....	129
Table 10. Nitrogen harvest index (NHI) of common bean ( <i>Phaseolus vulgaris</i> L.) plants grown under irrigated (nonstressed) and rainfed (stressed) moisture regime in a 1999 and 2000 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. The NHI was computed as grams seed-N / grams total-N. N = 8 (combined), 4 (rainfed or irrigated).....	130
Table 11. Harvest index (HI) of nine common bean ( <i>Phaseolus vulgaris</i> L.) plants grown under irrigated and rainfed moisture regime in a 1999 and 2000 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. The HI was as gram seed DW / gram total DW. N = 8 (combined), 4 (rainfed or irrigated).....	131
Table 12. Nitrogen use efficiency (NUE) of common bean ( <i>Phaseolus vulgaris</i> L.) plants grown under irrigated and rainfed moisture regime in a 1999 and 2000 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. The NUE was computed as total g DW / total g N or g seed DW / g seed N. N = 36.....	132
Table 13. Nitrogen use efficiency (NUE) of nine common bean ( <i>Phaseolus vulgaris</i> L.) plants grown under irrigated and rainfed moisture regime in a 1999 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. The NUE was computed as total g DW / total g N or g seed DW / g seed N. g seed DW / g seed N. N = 8 (combined), 4 (rainfed or irrigated).....	133
Table 14. Nitrogen use efficiency (NUE) of nine common bean ( <i>Phaseolus vulgaris</i> L.) plants grown under irrigated and rainfed moisture regime in a 2000 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. The NUE was computed as total g DW / total g N or g seed DW / g seed N. g seed DW / g seed N. N = 8 (combined), 4 (rainfed or irrigated).....	134
Table 15. Nitrogen fixed (kg ha <sup>-1</sup> ) from common bean ( <i>Phaseolus vulgaris</i> L.) plants grown under irrigated and rainfed moisture regime in a 1999 and 2000 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. N-fixed was calculated by the difference method with DOR 364 (nn) and BAT 477 (nn) as the reference crops. N = 36.....	135

Table 16. Nitrogen fixed (kg ha<sup>-1</sup>) of nine common bean (*Phaseolus vulgaris* L.) genotypes grown under irrigated and rainfed moisture regime in a 1999 and 2000 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. N-fixed was calculated by the difference method with DOR 364 (nn) and BAT 477 (nn) as the reference crops. N = 8.....136

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with a  
condit  
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Figure  
taken a  
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## LIST OF FIGURES

- Figure 1. Transpiration rate (means  $\pm$  SE, n = 36) of nine genotypes of common bean grown under irrigated and rainfed moisture conditions at the Agricultural Experiment Station at the University of the Virgin Islands. Vertical bars indicate standard error of the mean at  $P \leq 0.01$ .....46
- Figure 2. Leaf temperature (means  $\pm$  SE, n = 36) of nine common bean genotypes using a LiCor (LI 1600 Steady State) porometer grown under irrigated and rainfed moisture conditions at the Agricultural Experiment Station at the University of the Virgin Islands. Vertical bars indicate standard error of the mean.....47
- Figure 3. Leaf temperature (means  $\pm$  SE, n = 8) of nine common bean genotypes taken with an infra-red thermometer at 57 DAP and grown under irrigated and rainfed moisture conditions at the Agricultural Experiment Station at the University of the Virgin Islands. Vertical bars indicate standard error of the mean at  $P \leq 0.10$ .....48
- Figure 4. Sentry probe counts (means  $\pm$  SE, n = 36) of nine common bean genotypes taken at a depth of 30.5 cm and grown under irrigated and rainfed moisture conditions at the Agricultural Experiment Station at the University of the Virgin Islands. Vertical bars indicate standard error of the mean, significant at 57 DAP at  $P \leq 0.10$ .....49

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## Literature review

### *Introduction*

Common bean (*Phaseolus vulgaris* L.) is grown on more than 12 million hectares and constitutes the most important food legume for more than 500 million people in Latin America, the Caribbean, and Africa (Laing et al., 1983). It is a major source of dietary protein throughout Latin America, the Caribbean and Eastern Africa, but per household consumption is declining as population increases outdistance production (Graham and Ranalli, 1997). Sixty percent of common bean production worldwide is grown under water stress, making drought the second largest contributor to yield reduction after disease (Singh, 1995). These constraints along with insect pests, heat stress, and low soil fertility (CIAT, 1981) have prevented the realization of the crop's yield potential and have caused production instability from one year to the next.

The physiological mechanisms that may help impart drought tolerance in common bean are still poorly understood. Carbon and nitrogen partitioning and remobilization, stomatal closure, osmotic adjustment, and root development may all be involved (Levitt, 1980; Kramer, 1983; Blum, 1985, 1988; Hale and Orcutt, 1987; Ludlow and Muchow, 1990; Foster et al., 1995). Plants are usually classified as drought resistant or drought susceptible based upon phenotypic plasticity and the level of yield reduction during water stress (Levitt, 1980; Hale and Orcutt, 1987). Rapid, inexpensive, and reliable methods for screening large amounts of germplasm would greatly aid efforts to develop drought resistant lines and a better understanding of plant metabolic processes would enable a

more efficient approach to germplasm improvement (Wortmann et al., 1998).

The conditions under which this annual, predominantly self-pollinated legume is grown are extremely variable. The diversity of conditions, coupled with highly specific local preferences for particular seed types or colors have complicated attempts at bean improvement (Graham and Ranalli, 1997). As a result, greatest progress has been made in breeding for the resolution of disease, insect and nutritional constraints, with only limited improvement in yield potential (Graham, 1978; Adams et al., 1985; Laing et al., 1985; Gepts, 1988a; and Schoonhoven and Voysest, 1991).

Inadequate soil nitrogen availability has also been identified as a major constraint to common bean production in Latin America and Africa (Wortmann et al., 1998). Unlike some legumes, common bean typically derives little of its nitrogen from the atmosphere under low input agriculture although  $N_2$  fixation can be substantial if soil phosphorus is adequate (Giller et al., 1998). Common bean is genetically variable in its ability to obtain nitrogen from the soil, for  $N_2$  fixation, and for partitioning of nitrogen (Graham, 1978; Rennie and Kemp, 1983).

### ***Diseases***

Diseases are the most important constraint to common bean production in Latin America and Africa (CIAT, 1981; Beaver, 1995). More plant pathogens and more virulent isolates of these pathogens exist in Latin America and Africa than in the temperate regions of North America and Europe (Beebe and Pastor-Corrales, 1991; Miklas et al., 1996). The prevalence and importance of each disease vary considerably with locality, season, year, and cultivar, however, some diseases such as ashy stem blight



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Ashy stem blight is caused by the fungus *Macrophomina phaseolina* (Tassi) Goid. (Dhingra and Sinclair, 1977). Ashy stem blight is a warm-temperature pathogen of the beans *P. vulgaris* and *P. lunatus* L., soybean (*Glycine max* L.), maize (*Zea mays* L.), sorghum (*Sorghum bicolor*), and many other crops (Watanabe et al., 1970). The disease occurs mainly in Latin America but also in other parts of the world such as Kenya, Zambia, and Egypt (CIAT, 1981; Stoetzer, 1984). The disease is more prevalent and damaging to common bean that are exposed to drought and warm temperatures (CIAT, 1989). There seems to be a relationship between ASB resistance and drought tolerance. Two lines of *P. vulgaris*, BAT 477 and San Cristobal 83, appear to have both ASB resistance and drought tolerance traits (personal communication, Dr. James Beaver).

Common bacterial blight (CBB), a systemic (Burkholder, 1921), seed-transmitted (Aggour et al., 1989b) disease caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye (*Xcp*) (Saettler, 1989; Schuster and Coyne, 1981) frequently and severely attacks common bean grown in the tropics and subtropics (Singh and Munoz, 1999). Common bacterial blight is widespread in Latin America, particularly in northwestern Argentina, south central Brazil, Venezuela, Central America and Cuba, and coastal Mexico (Singh and Munoz, 1999). Common bacterial blight attacks all aerial plant parts, including leaf petioles, pods, and seeds, but the characteristic symptoms of chlorotic borders and necrotic lesions are more severe and conspicuous on leaves of susceptible cultivars (Singh and Munoz, 1999). Common bacterial blight can survive for months on plant debris left on the soil and in seeds (Gilbertson et al., 1990). Heavy and early infection,

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high humidity, temperatures fluctuating between 20 and 25°C, and alternately dry and wet weather can cause more than 40% yield loss in susceptible cultivars (Serracin et al., 1991). Other factors influencing disease severity are photoperiod (Arnaud-Santana et al., 1993a), inoculation method, source and type of inoculum, and bacterial concentration (Aggour et al., 1989a), and stage of crop maturity at infection (Coyne and Schuster, 1974).

### ***Drought***

White and Singh (1991) estimated that more than 60% of common bean grown in Latin America, Africa, and Asia suffer from water stress during crop growth. In Latin America alone, where one third of the world's common bean are produced, 93% of the common bean growing areas experience moisture stress (Fairbairn, 1993). The intensity of drought stress and the phenological stage of development at which drought occurs is unpredictable and differs for each year and region. Thus, moisture stress influences crop yield in different ways in different regions (Acosta-Gallegos and Adams, 1991).

Common bean are particularly susceptible to drought during flowering, with significant flower and pod abortion occurring when water shortage occurs at this time (Graham and Ranalli, 1997). Nunez-Barrios (1991) observed in common bean that water deficit hastened flowering and seed fill but delayed leaf appearance. Rapid root expansion was noted at the beginning of the water deficit period, and was followed by root death and compensatory growth in deeper soil layers. Drought may be terminal, where there is a gradual decrease of soil moisture as the plant matures, or intermittent in which moisture stress persists for seven days or longer. Intermittent stress may occur in

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less than 7 days on coarse textured soils in the tropics (personal communication, Dr. James Beaver) and may occur once or several times in the growing season (Levitt, 1972).

Drought resistance is defined by Hall (1993) as the relative yield of a genotype compared to other genotypes subjected to the same drought stress. Drought resistance in some species has been clearly demonstrated by the work on corn (*Zea mays*), sorghum, wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and bean (Begg and Turner, 1976; Morgan, 1984; Turner, 1986; Acevedo, 1987; Singh, 1989). Species differences in drought resistance depend on the type of economic product of the species (Hall, 1993). Species producing leafy vegetables, such as lettuce (*Lactuca sativa*), have little drought resistance, and tuber crops, such as potato (*Solanum tuberosum*), are more resistant to drought than leafy vegetables, but their yield and quality can be reduced by mild or moderate drought (Hall, 2001). In contrast, hay crops such as alfalfa (*Medicago sativa* L.) are even more drought resistant, and their yield is only reduced when drought becomes moderate and where economic yield is a reproductive organ (Hall, 2001). Resistance to drought depends on the stage of reproductive development, the type of economic product, and whether the plant is determinate or indeterminate (Hall, 2001).

The mechanisms of drought resistance in crop plants have been divided into several categories: drought escape, dehydration avoidance, dehydration tolerance, feedforward responses, and water use efficiency (Kramer, 1980, 1983; Levitt, 1980; Turner, 1986; Blum, 1988; Ludlow and Muchow, 1990; Hall, 2001). Drought escape is the ability of a plant to escape drought by completing its life cycle during the favorable moisture conditions prior to the onset of drought. Drought escape or evasion has

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sometimes been incorrectly equated to drought avoidance (Levitt, 1980; Blum, 1988).

Dehydration avoidance is the ability of a plant to prevent water loss by stomatal closure resulting in the maintenance of turgor during periods favoring high rates of transpiration.

Dehydration tolerance is the ability of a plant to withstand injury when plants are under drought stress. Feedforward response (Hall, 2001), is the theory that roots sense difficult conditions in the soil and send signals to the shoot that cause partial stomatal closure and slow down leaf expansion before the supply of water or nutrients is affected (Passioura and Stirzaker, 1993). Water use efficiency is the ratio of biomass production to transpiration.

### ***Roots***

Roots play an important role in the growth and survival of plants during periods of drought stress. Under drought, the root is characterized by a low root density in the dry surface layer and a higher root proliferation in the deeper, wetter soil layers (Smucker et al., 1991). Under non-stress conditions, roots proliferated in the soil zone with the lowest soil water retention (Garay and Wilhelm, 1983). A root system that extends the root zone to more fully extract available soil water has the potential to increase yield under drought (Mambani and Lal, 1983.). Thus, water uptake and transport by roots are very important, especially under water limiting conditions (Nguyen et al., 1997).

In common bean, differences in plant growth habit are mirrored by differences in root morphology. Type II growth habit is characterized by an intermediate, upright plant structure with reduced branching angle whereas type III habit is typical of an intermediate prostrate sprawling plant structure (Brothers and Kelly, 1993). Type II plants develop a



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thick tap root which can exploit deeper soil levels where water is often stored and type III plants, exhibit a shallow expansive root morphology (Lynch and van Beem, 1993).

When the plant root is to be defined for breeding and genetic transformation work, it must be recognized that the root can be described on the basis of its potential traits or on the basis of its stress-induced dynamic response (Nguyen et al., 1997). When drought stress develops, the root/shoot (R/S) ratio increases (Creelman et al., 1990; Leskovar and Cantliffe, 1992). Most certainly root morphology and distribution change. These changes may have a genetic basis and are the integrated expression of various adaptive processes taking place in the root in response to plant water deficit and a drying soil (Nguyen et al., 1997). Overall, the root traits of water uptake and root length have been studied by many researchers and have strong potential for improvement through breeding with the major limitation being the labor intensive screening for most root traits (Ingram et al., 1994).

Root development and capacity of plants to absorb water are closely related. As root width, depth, and branching increased, plant water stress decreased (Hurd, 1976). Levitt (1972) observed that when ground water was available, deep rooted plants showed greater drought avoidance than shallow rooted ones but they showed lower avoidance when deeper soil moisture was not present. Rooting depth and resistance to water flow within the root were important attributes of root systems when plants were grown in drought-prone environments (Taylor, 1980). White et al. (1990) reported that drought resistance in common bean was related to rooting depth.

Root architecture may also be important for mining minerals, nutrients, and water

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from the soil (Lynch and Van Beem, 1993). Fitter (1991) developed topological indices to quantify root architecture in two-dimensions, ranging from a herringbone structure at one extreme to a highly branched, dichotomous structure at the other extreme. Based on comparisons of ecologically distinct species and simple modeling exercises, Fitter (1991) proposed that root architecture may influence the efficiency of plant nutrient uptake.

Soil exploration by roots was associated with nutrient acquisition, especially in the case of immobile nutrients such as phosphorus (Lynch and Van Beem, 1993). Genetic differences in common bean were reported for root biomass, R/S ratio (Hannah et al., 2000; Borch et al., 1999; Fawole et al., 1982; Stoffela et al., 1979a), and for root biomass distribution among distinct root types (Stoffela et al., 1979b).

Some researchers have shown that the ability of a rice (*Oryza sativa* L.) plant to reach deep soil moisture or to penetrate compacted soils was linked with the capacity of the plant to develop a few thick (lateral) and long root axes (Yoshida and Hasegawa, 1982; Ekanayake et al., 1985; Ingram et al., 1994; Yu et al., 1995). Thick roots persisted longer and produced more and larger branch roots, thereby increasing root length density (RLD, defined as the total root length divided by the volume of soil occupied by the root) and water uptake (Fitter, 1991; Ingram et al., 1994).

#### ***Nitrogen Fixation and its effect on drought resistance***

Nitrogen is the major limiting nutrient required for plant growth, especially in agricultural systems (Date, 1973). It is an important component of the biochemical constituents that enhance yield producing processes (Sinclair and Horie, 1989). However, it is unclear whether moisture stress increases or decreases the sensitivity of

plants to nitrogen deficiency (Bennett et al., 1989). Plants in soils with low nitrogen have reduced growth rates and a low root to shoot (R/S) ratio (Russel, 1977).

Common bean is considered to be an inefficient nitrogen fixer and requires N fertilizer (Westermann et al., 1981). Inefficient nitrogen fixation in common bean is mostly caused by the failure to establish efficient symbiosis in the field. Common bean begins to fix nitrogen at a considerably later vegetative stage than other legumes, such that periods of nitrogen stress are observed in common bean before nodules begin to actively fix nitrogen (Westermann et al., 1981). To avoid periods of nitrogen stress in the field, a starter fertilizer of N (40 kg ha<sup>-1</sup>) is usually applied (Sprent and Thomas, 1984).

The effect of water stress on nitrogen fixation, accumulation, partitioning, and remobilization in common bean is well documented (Ramos et al, 1999; Serraj and Sinclair, 1998; Castellanos et al., 1996; DeVries et al., 1989). Moisture stress affects the total accumulation of nitrogen in many species, including cowpea (*Vigna unguiculata* (Walp) L.), soybean, and common bean (Chapman and Muchow, 1985). Water stress affects rhizobial survival and growth in soil, the formation and longevity of nodules, synthesis of leghemoglobin and nodule function and is a major cause of nodulation failure and low N<sub>2</sub> fixation (Hungria and Vargas, 2000). Furthermore, severe water stress may lead to irreversible cessation of N<sub>2</sub> fixation (Sprent, 1971; Vincent, 1980; Walker and Miller, 1986; Venkateswarlu et al., 1989; Guerin et al., 1991). Foster et al. (1995) reported that a greater proportion of seed nitrogen was obtained from remobilized leaf nitrogen under moderate moisture stress conditions in common bean, but not under severe or prolonged moisture stress. Severe moisture deficits reduced N harvest index and N use

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efficiency. Foster et al. (1995) suggested that drought susceptible common bean genotypes may utilize nitrogen less efficiently than resistant genotypes.

Determinate, early maturing type I bush habit common beans fix the least nitrogen, while indeterminate climbing genotypes fix more nitrogen (Graham, 1978; Rennie and Kemp, 1983; Gardezi et al., 1990). Generally, early maturing varieties are inferior users of photosynthates for biological nitrogen fixation (Piha and Munns, 1987). However, it has been suggested that some common bean varieties (most likely type III) can acquire enough nitrogen either through fixation or assimilation of mineral nitrogen for the plants to achieve genetic yield potential under field conditions (Gardezi et al., 1990 Westermann et al., 1981).

Nitrogen fixation should be emphasized as the dominant N input in farming systems in the developing world , with fertilizer N usage in such systems focused on more highly productive cash crops (Hungria and Vargas, 2000). Kennedy and Cocking (1997) suggested that systems based upon N<sub>2</sub> fixation are most promising and potentially profitable in extensive rather than intensive agricultural systems, where erratic or historically low rainfall and market changes can seriously impact the economics and efficiency of fertilizer use. Appropriate soil management practices for the tropics (such as no-till) which results in decreases in soil temperature and increases in soil moisture, also benefit N<sub>2</sub> fixation (Graham and Vance, 2000).

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## Chapter 1

### **Common bean (*Phaseolus vulgaris* L.) yield under high pH, limited moisture, and low nitrogen.**

#### **Abstract**

In many regions, common bean (*Phaseolus vulgaris* L.) is grown under rainfed conditions where water deficits limit yield and cause instability of production. A field study was conducted in 1999 and 2000 to evaluate the effect of limiting moisture on seed yield. The study used a split plot arrangement in randomized complete block design with moisture as the main plot, genotypes as subplot, and four replications. Combined yield of the nine genotypes ranged from 142 to 1508 kg ha<sup>-1</sup> in 1999 and 568 to 896 kg ha<sup>-1</sup> in 2000. Mean yield of XAN 176, DOR 364 [nodulating (nod)], and PR9603-22 exceeded 1300 kg ha<sup>-1</sup>, despite infestations of common bacterial blight (*Xanthomonas campestris* pv. phaseoli), *Cercospora* (*Cercospora canescens*), bean leafskeletonizer (near *Autoplasia spp*), and ozone damage. In 1999, yield was reduced by 17 and 27% in the non-nodulating isolines of BAT 477 (nod) and DOR 364 (nod), respectively due to diseases. Days to flower (DF) ranged from 34 to 38 days after planting (DAP), and days to maturity ranged from 69 ( to 75 DAP. The geometric mean ranked PR9603-22, XAN 176, and DOR 364 (nod) among the top genotypes for drought resistance..

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## Introduction

Common bean (*Phaseolus vulgaris* L.) is the principal food legume of more than 500 million people in Latin America, Asia, and Africa and for more than 100 million of them, it is the leading source of dietary protein (CIAT, 1984) and an important source of calories. Production of common bean, in many regions, occurs under rainfed conditions where water deficit limits yield and causes instability of production (Ehleringer et al., 1991; White et al. 1994).

Common bean, a cheaper source of protein for developing countries in comparison to animal protein, (Singh and Jambunathan, 1981) has been reported to reduce the levels of cholesterol and blood glucose (Soni et al., 1982). There are also well recognized shortcomings in consuming animal proteins in the developing countries, such as unhygienic processing and storage and consequent microbial contamination (Singh and Singh, 1992).

Common bean yields have been low, averaging less than 1 ton ha<sup>-1</sup> in developing countries to 2 tons ha<sup>-1</sup> in developed countries (Laing et al., 1984; Adams, 1996). Yet yields of 2.19 to 4.12 tons ha<sup>-1</sup> are reported from experiment stations, indicating the enormous gap between the potential and actual yield for this crop. The most important production constraints in bean producing areas of the tropics are drought, diseases, insect pests, stress caused by low rainfall (moisture and heat), and low soil fertility (CIAT, 1984). These constraints limit yield and cause production instability from one year to the next. Drought is the major abiotic constraint, because almost all bean production in Latin America, the Caribbean, and Africa occurs on dryland farming systems with frequent

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water deficit affecting more than 60% of the crop produced (White et al., 1990; Laing et al., 1984).

The physiological mechanisms that help impart drought tolerance are still poorly understood. Carbon and nitrogen partitioning and remobilization, stomatal closure, osmotic adjustment, and root development may be involved (Kramer, 1980, 1983; Levitt, 1980; Turner, 1986; Blum, 1988; Ludlow and Muchow, 1990; Foster et al., 1995; Hall, 2001). Plants are usually classified as drought resistant or drought susceptible based upon phenotypic plasticity and the level of yield reduction during water deficit (Hale and Orcutt, 1987; Acosta-Gallegos, 1995).

Although agronomic practices are important under conditions of water deficit, cultivar improvement is usually seen as the most promising approach to increase yields under drought stress. Research has indicated that direct selection for seed yield in common bean can be effective, although time-consuming and costly, both for well-watered (Nienhuis and Singh, 1988; Singh et al., 1990) and water deficit conditions (White et al., 1994). Thus, the objective of this study was to determine the effect of limited moisture on seed yield in nine common bean genotypes grown under rainfed and irrigated conditions.

## **Materials and methods**

### ***Field study***

Two experiments were conducted at the Agricultural Experiment Station, University of the Virgin Islands, Kingshill, St. Croix, United States Virgin Islands U.S.V.I. (17° 42' N, 64° 48' W, and 33.5 masl) in 1999 and 2000. Mean temperature was

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26.1<sup>o</sup> C. Seeds were planted on 9 March and harvested on 1 June 1999 and on 6 April and harvested on 27 June 2000 (stress plots) and on 30 June 2000 (non-stress plots). The soil at the Experimental Station field site is classified as a Fredensborg loamy, fine carbonatic, isohyperthermic, shallow, Typic Calciustoll with pH ranging from 7.6 to 8.4.

In 1999, a soil sample from each plot were taken and analyzed by the Michigan State University Plant Nutrient and Soil Testing Laboratory for N, P, and K. Soil samples were also analyzed for Zn, Mn, and Cu. As indicated by the soil analysis, 22 kg P/ha<sup>-1</sup>, 5.6 kg Zn/ha<sup>-1</sup>, and 10 kg Mn/ha<sup>-1</sup> were applied in 1999 and 2000. No N fertilizer was applied, since N fixation was also being assessed. Samples from each block (stress and non-stress) were taken in 2000.

In 1999, applications of insecticide, Sevin 80WP (0.68 kg ai/A) and Diazinon AG500 (170 g ai/A), were made at one week intervals starting on 26 March to control the bean leafskeletonizer. One application of fungicide, Benomyl (500 g per 95 L/A) and M-Pede (Potassium salts of fatty acids) (71 g per 3.8 L/A) was made on 18 April for control of *Cercospora* (*Cercospora canescens*). No insecticides or fungicides were applied to field plots in 2000.

#### ***Water regime***

In 1999, 100.8 mm of rainfall were recorded during the growing season. In addition, plants were irrigated by drip irrigation on 31 days during the growing season for one hour on each date. Twelve applications were made before the initiation of stress at 21 DAP and 19 were made after stress initiation.

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Nine common bean genotypes possessing different Type I, II, and III growth habits (Table 1) were included in this study: BAT 477 [nodulating (nod) and non-nodulating (nn)], DOR 364 [nodulating (nod) and non-nodulating (nn)], XAN 176, ICA Palmar, 8-42-M-2, SEA5, and PR9603-22, local check (obtained from Dr. James Beaver, University of Puerto Rico-Mayaguez Campus). BAT 477 (nod) and 8-42-M-2 were the drought resistant and drought susceptible checks, respectively.

### ***Experimental design***

The study utilized a randomized complete block design with four replications, moisture as the main plot, and genotype as subplot. In 1999, seeds were planted into four-row plots of 0.5 m row spacing and 2.48 m length. Each row was planted at a density of 25 seeds and thinned to 23 plants. In 2000, seeds were planted into four-row plots of 0.5 m row spacing and 2.13 m length and planted at a density of two seeds per station at 7.62 cm between stations. Seeds were inoculated with a granular form of *Rhizobium etli*, which was applied directly within the seed station. Moisture stress was initiated at the V3 (Nuland and Schwartz, 1989) growth stage or 20 days after planting (DAP) by cessation of irrigation to the rainfed plots. Control plots were maintained at a soil moisture content of -30 kPa.

### ***Data collection***

Plots were sampled at vegetative (V3), flowering (R2), and podfill stages (R7) for N<sub>2</sub> fixation and weekly. Starting at 23 DAP, plants were sampled weekly for stomatal conductance, leaf temperature, and leaf transpiration. In 1999, plants were visually scored for disease on a scale of 0 to 9, with 9 being dead and 0 being no visual symptoms.

Days to flower (DF), defined as the number of days when 50% of the plants had one open flower; days to physiological maturity (DPM), defined as the number of days for 75 to 90% of the pods to lose their green pigmentation; and days to seed fill (DSF=DPM-DF) were calculated. In 1999, soil moisture was recorded at 44, 51, and 58 DAP using a Sentry 200-AP moisture probe (Troxler Electronics Laboratories, Inc.) and in 2000, soil moisture was determined using the gravimetric method. A hard soil pan prevented soil moisture recordings below 30 cm, consequently, soil moisture was only recorded at a depth of 30 cm. At harvest, seed yield was determined at 18% moisture. The MSTAT micro-computer statistical package (Michigan State University) for agricultural sciences was used for all data analysis.

#### ***Moisture stress indices***

Geometric mean (GM) separates genotypes into four categories: (1) those that yield well both under stress and non-stress environments, (2) those that yield well only under non-stress, (3) those yielding relatively well under stress, and (4) those yielding poorly under both stress and non-stress conditions (Fernandez, 1993). The GM is calculated as:  $GM = (Y_s * Y_p)^{1/2}$ . Where  $Y_s$  = the yield of a given genotype in a stress environment and  $Y_p$  = the potential yield of a given genotype in a non-stress environment. Fernandez (1993) and Schneider et al. (1997) observed that the choice of GM to represent mean productivity is preferred because, when ranking genotypes, GM better accounts for large differences in performance between stress and non-stress environments than does the simple arithmetic mean of stress and non-stress yields used by Rosielle and Hamblin (1981).



The drought susceptible index (DSI) is reported to estimate drought tolerance. A value of one is reported to equal average resistance, values lower than one represent greater than average resistance, and values greater than one indicate susceptibility (Fischer and Maurer, 1978). The DSI of individual genotypes is calculated as:  $DSI = [1 - (Y_s / Y_p)] / DII$ , and was the index preferred by Ramirez and Kelly (1998).

The drought intensity index (DII) is calculated as:  $DII = [1 - (Y_s / Y_p)]$ . Where  $Y_s$  = mean yield in stress environment and  $Y_p$  = mean yield in non-stress environment (Fernandez, 1993). It ranges between 0 and 1 and the larger the value of DII, the more severe the stress intensity of the test.

The stress tolerance index (STI) has been developed as an alternative to the DSI. Stress tolerance index is reported to measure both stress tolerance and yield potential (Fernandez, 1993). With STI, the higher the value, the greater the stress tolerance and the higher the yield. Genotypes chosen based upon high STI exhibit high yield potential and high yield in stress environments (Fernandez, 1993). The STI is calculated as:  $STI = [(Y_s)(Y_p)] / (Y_p)^2$ .

## **Results and discussion**

### **1999**

Soil pH across all plots ranged from 7.6 to 8.0. Soil iron (Fe) content ranged from 3 to 8 ppm with a mean of 5 ppm and percent organic matter ranged from 1.96 to 2.61 with a mean of 2.35. In 1999, soil  $NO_3^-$  ranged from 11 to 39 ppm with a mean of 24 ppm and soil  $NH_4$  ranged from 2 to 6 ppm with a mean of 4 ppm.

The genotypes ICA Palmar and PR9603-22 flowered at 34 DAP, while the other

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genotypes flowered at 38 DAP. However, ICA Palmar never reached physiological maturity (Table 2). The other genotypes matured over a range of 69 to 75 DAP, and DSF ranged from 35 to 37 days (Table 2). There were no significant differences among stress and non-stress treatments (Figure 1) or among genotypes (data not shown) for transpiration rate. Likewise, leaf temperature measured using a Li-Cor (LI-1600 Steady State Porometer) porometer was not significant among stress and non-stress treatments on any sampling dates (Figure 2), however, at 57 DAP using an infrared thermometer, there were significant genotypic differences with the genotype ICA Palmar having a significantly higher ( $P \leq 0.10$ ) leaf temperature than the other genotypes (Figure 3). Precipitation was higher than normal (Appendix A), so moisture stress was mild and soil moisture did not differ between rainfed and control plots except at 58 DAP (Figure 4).

Yield under irrigated conditions ranged from 151 to 1478 kg ha<sup>-1</sup> and for rainfed conditions from 142 to 1801 kg ha<sup>-1</sup> (Table 3). Under irrigated conditions, the genotype DOR 364 (nod) had a significantly higher ( $P \leq 0.01$ ) seed weight than ICA Palmar and 8-42-M-2 but not significantly higher than the other genotypes (Table 3). In the rainfed treatment, the genotype PR9603-22 had a significantly higher ( $P \leq 0.001$ ) seed weight than the genotypes, ICA Palmar, BAT 477 (nn), SEA5, and 8-42-M-2 (Table 3). The local check, PR9603-22 performed well under both irrigated and rainfed conditions and the data for PR9603-22 agreed with previous results obtained at the University of Puerto Rico-Mayaguez indicating that it is a high yielding bean genotype (Personal communication, Dr. James Beaver). Combined yield of the nine genotypes ranged from 142 to 1508 kg ha<sup>-1</sup>, number of pods per m<sup>2</sup> from 64 to 519, and pod weight per m<sup>2</sup> from

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17 to 132 grams (Table 3). DOR 364 (nod), XAN 176, and the local check PR9603-22 had a significantly higher ( $P \leq 0.01$ ) combined yield than the genotypes ICA Palmar, BAT 477 (nn), SEA5, and 8-42-M-2 but not significantly higher than BAT 477 (nod) and DOR 364 (nn). Differences in number of pods per  $m^2$  and pod weight per  $m^2$  were highly significant among the genotypes ( $P \leq 0.01$ ) and among water treatments ( $P \leq 0.05$ ) but not for genotype x stress. The genotypes DOR 364 (nod), XAN 176, and DOR 364 (nn) had a significantly ( $P \leq 0.01$ ) higher number of harvested pods than BAT 477 (nod & nn), ICA Palmar, SEA5, and 8-42-M-2 (Table 3). The genotype ICA Palmar had a significantly lower ( $P \leq 0.01$ ) pod weight per  $m^2$  and lower number of pods harvested per  $m^2$  than all the genotypes (Table 3), because ICA Palmar did not reach physiological maturity. ICA Palmar is a Type I bean genotype (Haley et al., 1994) and has a determinate bush growth habit. Beaver et al. (1985) found that determinate bean genotypes tend to have lower yield potential and have less yield stability than indeterminate bean genotypes, although ICA Palmar performed well in the winter nursery in Mayaguez, Puerto Rico (Beaver and Kelly, 1994).

There was no significant difference between the resistant check, BAT 477 (nod) (CIAT, 1984; Gregory, 1989), and the susceptible check, 8-42-M-2 (Acosta-Gallegos, 1988; Manthe, 1994; Yabba, 1997), for yield under irrigated or rainfed treatments and for combined yield (Table 3). The resistant check, BAT 477 (nod) had a significantly higher ( $P \leq 0.01$ ) number of pods per plot and a significantly higher pod weight per plot than 8-42-M-2 (Table 3). The susceptible check, 8-42-M-2, produced 64% more seed under rainfed conditions compared to the irrigated treatment and BAT 477 (nod) produced 27%

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more seeds under rainfed conditions compared to the irrigated conditions. Both genotypes produced a greater yield under rainfed than irrigated, hence, yield reduction for these two genotypes were negative. The resistant check, BAT 477 (nod) was one of the better performers in 1999.

DOR 364 (nn), ICA Palmar, BAT 477 (nn), and SEA5 were the only genotypes to have a yield reduction under rainfed conditions (Table 3) with the genotype ICA Palmar having the highest percent yield reduction. BAT 477 (nn) and DOR 364 (nn) had a 27 and 17% yield reduction, respectively, in comparison to their nodulating isolines. The drought susceptible index and the STI selection criteria for assessing plant moisture stress tolerance, were not calculated for 1999 because yield from five of the nine genotypes in the stress plots out-yielded plots from the non-stress treatment. However, GM ranked the top four genotypes as DOR 364 (nod), PR9603-22, XAN 176, and DOR 364 (nn) (Table 3).

Plants were severely infected with common bacterial blight (*Xanthomonas campestris* pv. *Phaseoli*), *Cercospora* (*Cercospora canescens* Ellis & G. Martin), bean leafskeletonizer (near *Autoplusia* spp), and ozone damage and N-deficiency. ICA Palmar had a significantly higher ( $P \leq 0.01$ ) rating for common bacterial blight than all other genotypes (Table 4), supporting other work (CIAT, 1979), indicating ICA Palmar's susceptibility to common bacterial blight. DOR 364 (nod & nn), PR9603-22, and XAN 176 had the lowest common bacterial blight rating and were the highest yielding lines, reflecting the significant relationship ( $R^2 = 0.34$ ,  $P \leq 0.001$ ) between common bacterial blight and yield. Common bacterial blight affects the foliage and pods of beans and is

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considered to be a major problem in most bean production areas of the world (Hall, 1994). During extended periods of warm, humid weather, the disease can be highly destructive, causing losses in both yield and seed quality. Common bacterial blight typically develops from (1) planting contaminated seeds, (2) planting seeds in a contaminated field, and (3) when the climate is consistently hot and wet or humid (Hall, 1994). Because clean seed was planted, common bacterial blight must have been preexisting in the soil and was brought on by hot and wet weather conditions throughout the growing season.

Bean leafskeletonizer infected the plants but was controlled with the insecticides Sevin and Diazinon. *Cercospora* (*C. canescens* & *C. cruenta*) occurs in Latin America and the southern United States. It can affect all aerial parts of common bean and result in defoliation. The *Cercospora* rating for SEA5 was significantly higher ( $P \leq 0.01$ ) than that of all other genotypes, and ozone damage was significantly greater ( $P \leq 0.05$ ) on PR9603-22 than on all other genotypes except BAT 477 (nod) and SEA5 (Table 4). There was no significant water x genotype interactions for *Cercospora* and common bacterial blight but there was for ozone damage. Significant water x genotype interaction occurred within the ozone count rating (Table 4). The genotype PR9603-22 had the highest ozone rating in both irrigated and rainfed treatments (Table 4). Although the genotype PR9603-22 showed the highest ozone rating, it was still one of the higher yielding genotypes in the study. As with common bacterial blight, DOR 364 (nod & nn), PR9603-22, and XAN 176 had the lowest *Cercospora* rating and were among the highest yielding lines.

The genotype, ICA Palmar produced yields exceeding 2000 kg<sup>-1</sup> ha at the Agricultural Research Experiment Station in Isabella, Puerto Rico, demonstrating its high yield potential (Personal Communication, Dr. James Beaver). The failure to mature, the severe common bacterial blight infestation, the mild infestation of *Cercospora*, ozone damage, and feeding damage from the bean leaf skeletonizer were significant contributors to the low yield of ICA Palmar obtained at St. Croix. The failure of ICA Palmar to mature is inexplicable because there is no difference in photoperiod between Isabella, Puerto Rico and St. Croix. Further investigation is needed to assess its response to high pH and soil and air temperature.

Previous work with SEA5 (Singh (1995) and Singh et al.(2001) and BAT 477 (nod) in Mexico (Personal Communication with Dr. Jorge Acosta) produced yields that were appreciably higher than the ones obtained in this study. Nevertheless, the average yield obtained in 1999 was greater than the average yield obtained in many areas of the Caribbean and demonstrate the adaptability of common bean to St. Croix and the ability to produce competitive yields despite insect and disease problems.

### **2000**

In 2000, soil NO<sub>3</sub><sup>-</sup> ranged from 20 to 42 ppm with a mean of 30 ppm and soil NH<sub>4</sub> ranged from 4 to 21 ppm with a mean of 9 ppm. In 2000, yield was recorded for eight genotypes. The genotype ICA Palmar was dropped from yield analysis because of failure to mature in 1999. There were no significant differences among the genotypes for rainfed treatment, irrigated treatment, and combined yield (Table 5). Also, there was no genotype x stress interaction. Irrigated yield ranged from 719 to 1291 kg ha<sup>-1</sup>, rainfed

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yield ranged from 418 to 743 kg ha<sup>-1</sup>, and combined yield ranged from 568 to 896 kg ha<sup>-1</sup>. Combined yield was considerably lower than combined yield obtained in 1999. In 2000, plants showed visible signs of nitrogen deficiency, contributing to the low yield observed. The genotype PR9603-22 produced moderately good yield under both treatments. Yield reduction among the genotypes ranged from 15% (SEA5) to 66% (XAN 176) (Table 5).

Number of pods ranged from 102 to 220, and pod weight from 53 to 75 grams (Table 5). The genotype 8-42-M-2 produced a significantly lower ( $P \leq 0.01$ ) number of pods than all genotypes except the genotypes PR9603-22, BAT 477 (nn), and SEA5, however pod weight, fifty seed weight, and seeds per pod did not differ among the genotypes. There were significant differences within irrigated and rainfed treatments in the number of pods harvested ( $P \leq 0.01$ ), pod weight ( $P \leq 0.001$ ), and fifty seed weight ( $P \leq 0.10$ ) but not for number of seeds per pod (Table 5).

Geometric mean was used to assess yield potential, an important factor since a genotype might be low yielding under sufficient moisture conditions, but have minimal yield reduction under stress. The GM ranked DOR 364 (nn), PR9603-22, SEA5, and DOR 364 (nod) in that order, as having the highest yield potential (Table 5). The genotype XAN 176 had the highest DSI and SEA5 had the lowest (Table 5). According to this system, the resistant genotypes in order from most to least resistance were SEA5, PR9603-22, DOR 364 (nn), and BAT 477 (nod). The genotype PR9603-22, DOR 364 (nod), and DOR 364 (nn) were among the top four genotypes selected by GM in 1999 and in 2000. The susceptible genotypes in order from most to least susceptible were XAN 176, BAT 477 (nn), DOR 364 (nod), and 8-42-M-2.

Stress tolerance index ranged from 0.30 to 0.77 with the genotype DOR 364 (nn) having the highest value indicating the greatest resistance and highest yield potential and the genotype 8-42-M-2 having the lowest value indicating susceptibility and low yield potential (Table 5). According to the STI, the genotypes having the greatest resistance and highest yield potential were DOR 364 (nn), PR9603-22, SEA5, and DOR 364 (nod), similar to results obtained for GM. Stress tolerance index and DSI agreed on the genotypes that would be assessed as resistant or susceptible, but the order within categories differed.

The GM, DSI, and STI were each analyzed to determine their degree of correlation with yield under stress conditions, yield under non-stress conditions, and combined yield of the two moisture treatments. The correlation of GM and STI with yield under stress and combined yield was positive and highly significant, ranging from 0.79\* to 0.94\*\*\* (Table 6). As expected, the DSI was inversely correlated with yield under stress (-0.83\*\*) (Table 6). The GM and STI were more accurate than the DSI in selecting desirable genotypes based upon yield performance at the Agricultural Experiment Station in 2000. Our results are similar to results obtained by Schneider et al. (1997) who concluded that GM was the single strongest indicator of yield performance under stress and non-stress. They suggested that the most effective breeding strategy to improve drought resistance in common bean should first involve selection based on the GM, followed by selection based on yield under stress.

#### ***Effect of pH on yield of common bean***

The high soil pH (7.6 to 8.0) at the Agricultural Experiment Station caused

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concern about its potential effect on seedling germination rate and on yield since common bean grows best at a pH range of 6.0 to 7.2 (Hall, 1994). In 1999, there was a germination rate of 90% and in 2000 of approximately 85%. In 2000, due to limited water pressure at the Agricultural Experiment Station, germination on the South side of the trial (approximately 3 plots from each replication and treatment) was sporadic and those plots had to be replanted. Mean seed yield for 1999 was 1100 kg ha<sup>-1</sup> (excluding the genotype ICA Palmar, 994 kg ha<sup>-1</sup> including ICA Palmar) and 803 kg ha<sup>-1</sup> for 2000, excluding the genotype ICA Palmar. These yield are comparable to results obtained in Trinidad (1100 kg ha<sup>-1</sup>, Gonsalvez, 1975), but low compared to reports from other bean growing areas in the Caribbean such as Puerto Rico (1988; 2100 kg ha<sup>-1</sup>, Badillo-Feliciano, 1977), the Dominican Republic (1700 kg ha<sup>-1</sup>, Beaver et al., 1988), Jamaica (1300 kg ha<sup>-1</sup>, Malcolm and Salmon, 1979), and Cuba (1362 kg ha<sup>-1</sup>, Isasi and Busto, 1984). Results from the two years of this study showed that seedling germination and growth of common bean is possible on a high pH soil at the Agricultural Experiment Station and that yields, while low, are still competitive with a few other bean producing areas of the Caribbean.

The most prominent nutritional disorders of crop plants grown in soils with high pH are iron, zinc, and manganese deficiencies (Schinas and Rowell, 1977). Plant species that are mainly affected include apple (*Malus domestica* Borkh.), peaches (*Prunus persica* (L.) Batsch.), grape (*Vitis vinifera* L.), peanut (*Arachis hypogaea* L.), soybean (*Glycine max* (L.) Merr.), sorghum (*Sorghum bicolor* L.), and upland rice (*Oryza sativa* L.) (Marschner, 1997). It is the major problem in sorghum and soybean production in the

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Great Plains of the United States (Clark, 1988). Iron deficiency chlorosis is frequently observed in beans grown on high pH calcareous soils where there is a decrease in the iron solubility due to the formation of insoluble ferric oxides (Lindsay and Schwab, 1982). Severe iron deficiency chlorosis can cause significant seed yield reduction in common bean (Zaiter et al., 1992). Reports indicated that common beans have a high sensitivity to iron deficiency (Clark, 1988). Symptoms of iron deficiency in common bean appear in young leaves, which become pale yellow, almost white, while the veins remain green, fully expanded leaves curve downward, and leaf tips may wilt (Hall, 1994). Similar symptoms were visually observed in the field trials in 1999.

#### ***Nutritional and pathological problems***

In 1999, 52% ( $r^2 = 0.52$ ,  $P \leq 0.01$ ) of the yield under irrigated conditions was explained by plant response to CBB ( $r = -0.35$ ,  $P \leq 0.05$ ) and potassium ( $r = -0.14$ ,  $P \leq 0.001$ ) while 42% ( $r^2 = 0.42$ ,  $P \leq 0.01$ ) of the yield under stress conditions was explained by CBB ( $r = -0.32$ ,  $P \leq 0.01$ ) and ozone damage ( $r = 0.23$ ,  $P \leq 0.05$ ). Field plots were not analyzed for iron in 1999, but an iron test was performed on soil samples in 2000. Iron concentration at the UVI Agricultural experiment Station ranged from 4 to 8 ppm (0.004 to 0.008 mg Fe kg<sup>-1</sup> of soil) which is low, since mineral soils have, on average, a total iron content of approximately 2% (20 mg Fe kg<sup>-1</sup> of soil) (Marschner, 1997). The data and visual symptoms suggest that plants suffered from iron deficiency in 1999 and 2000. Furthermore, plants displayed visual symptoms of N deficiency, especially in 2000; *Macrophomina phaseolina* and *Rhizoctonia solani* in 2000, and common bacterial blight and *Cercospora* in 1999 and 2000.

## Conclusion

Days to flower ranged from 34 to 38 DAP and days to maturity ranged from 69 to 75 DAP. Yield at the University of the Virgin Islands Agricultural Experiment Station for the nine genotypes in this trial ranged from 142 to 1508 kg ha<sup>-1</sup> in 1999, and from 568 to 896 kg ha<sup>-1</sup> in 2000. Yields were severely reduced by a combination of factors such as high pH, nitrogen deficiency, common bacterial blight, Cercospora, ozone damage, and bean leafskeletonizer. The genotypes ICA Palmar and SEA5 had the greatest yield reduction due to these diseases, each producing less than 700 kg ha<sup>-1</sup>. However, XAN 176, DOR 364 (nod), and the line PR9603-22 produced yields exceeding 1300 kg ha<sup>-1</sup>. These lines exhibited a higher tolerance to moisture stress and showed that relatively high yields are possible in St. Croix despite high soil pH, shallow alkaline soils, and insect and disease problems. Results are important because Crucians consume large quantities of common bean and the island has the potential for common bean production, although none is grown on the island. Future work should investigate bean pathogens and nutritional disorders.

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Table 1. Characteristics of common bean genotypes grown in field experiments at the Agricultural Experiment Station (University of the Virgin Islands) St. Croix, United States Virgin Islands (U.S.V.I.) in 1999 and 2000.

Genotypes	Parent	†Origin	Where developed	‡Plant type	§Seed size	Seed color	Comments
BAT 477 (nod)	(51051 / ICA Bunsi) / (51012 / Cornell 49-242)	M	CIAT	II	M	Brown	Resistant check
DOR 364 (nod)	BAT 1215 // RAB 166 / DOR125	M	ICTA / CIAT	II	S	Red	Drought resistance
XAN 176	BAT 304 / XAN 87	M	CIAT	II	S	Black	To be evaluated
BAT 477 (nn)	(51051 / ICA Bunsi) / (51012 / Cornell 49-242)	M	CIAT	II	M	Brown	Reference crop
DOR 364 (nn)	BAT 1215 // RAB 166 / DOR125	M	ICTA / CIAT	II	S	Red	Reference crop
ICA Palmar	Linea 11 x Huila 27	A	ICA/CIAT	I	ML	Red	Drought resistance
SEA 5	BAT 477 (nod) / San Cristobal 83 // Guanajuato 31 / Rio Tibagi	M	CIAT	III	S	Cream colored	Ashy stem blight resistance
8-42-M-2	N81017 X LEF-2-RB	M	MSU	III	M	Tan or Brown	Susceptible check
PR9603-22	PR9156-61 / DOR 482	M	UPR	II	M	Pink	Local check

† Indicates Andean (A) or Mesoamerican (M) types.

‡ Indicates Type I, Type II, or Type III.

§ Indicates large (L), medium (M), or small (S).

UPR University of Puerto Rico.

MSU Michigan State University.

CIAT International Center for Tropical Agriculture.

ICTA Institute for Science and Agricultural Technology.

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Table 2. Days to flower (DF), days to physiological maturity (DPM), and days to seed fill (DSF) of nine common bean (*Phaseolus vulgaris* L.) genotypes grown at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix Campus, U.S.V.I.

Genotypes	DF	DPM	†DSF
BAT 477 (nod)	38	75	37
PR9603-22	34	69	35
DOR 364 (nn)	38	73	35
ICA Palmar	34	‡	--
XAN 176	38	73	35
BAT 477 (nn)	38	75	37
SEA5	38	75	37
8-42-M-2	38	75	37
DOR 364 (nod)	38	73	35

† DSF = DPM - DF

‡ The genotype ICA Palmar did not reach physiological maturity.

**Table 3. Yield under stress and nonstress treatments (kg ha<sup>-1</sup>), combined yield (kg ha<sup>-1</sup>), percent yield reduction, geometric mean (GM), number of pods harvested per plot, and pod weight per plot (g), of nine common bean (*Phaseolus vulgaris* L.) genotypes grown at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix Campus, U.S.V.I. 1999. N = 4 (rainfed and irrigated yield) and 8.**

Genotypes	Irrigated yield (kg ha <sup>-1</sup> )	Rainfed yield (kg ha <sup>-1</sup> )	†Comb. yield (kg ha <sup>-1</sup> )	‡% Yield Difference	GM (kg ha <sup>-1</sup> )	#Pods/ m <sup>2</sup>	Pod wt./m <sup>2</sup> (g)
BAT 477 (nod)	871 abc**	1201 abc***	1036 abc**	-27	1022	181 cde**	55.24 ab**
PR9603-22	1146 ab	1807 a	1476 a	-37	1439	205 bcd	65.68 a
DOR 364 (nn)	1318 ab	1193 abc	1256 abc	9.5	1254	243 abc	68.81 a
ICA Palmar	157 c	126 d	142 d	20	140.8	35 g	9.24 d
XAN 176	1190 ab	1603 ab	1397 ab	-26	1382	266 ab	71.46 a
⊕ BAT 477 (nn)	775 abc	737 cd	756 bcd	5	755.4	174 def	47.40 bc
SEA5	681 abc	586 cd	633 cd	14	631.6	122 ef	40.00 c
8-42-M-2	393 bc	1085 bc	739 bcd	-64	652.6	111 f	34.76 c
DOR 364 (nod)	1478 a	1539 ab	1508 a	-4	1508	281 a	72.11 a
Mean	890	1097	994	-19	—	179	51.42

† Indicates combined stress and non-stress yield.

‡ Indicates yield difference between irrigated and rainfed environments. The value is positive if irrigated treatment has the higher yield and negative if rainfed treatment had the higher value.

\*\*\*, \*\* Different letters indicates significant difference among means within a column at P ≤ 0.001 and 0.01, respectively, according to DMRT.

Table 4. Common bacterial blight (CBB) (*Xanthomonas campestris* pv. *phaseoli*), *Cercospora* (*Cercospora canescens*), and ozone rating of nine bean (*Phaseolus vulgaris* L.) genotypes grown at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix Campus, U.S.V.I. 1999. Scale 0 to 9 with 0 = no visual symptoms and 9 = death. N = 8 and 4 (rainfed and irrigated treatments).

Genotypes	CBB		Cercospora		Ozone	‡Ozone	§Ozone			
BAT 477 (nod)	4.75	b**	6.20	b**	3.63	ab*	4.50 ¶abc+	2.75	d	
PR9603-22	2.36	c	2.75	cde	4.88	a	5.00	a	4.75	ab
DOR 364 (nn)	2.19	c	2.31	de	3.38	b	2.25	d	4.50	abc
ICA Palmar	8.25	a	3.50	cd	2.88	b	2.50	d	3.25	bcd
XAN 176	2.50	c	2.31	de	2.44	b	2.63	d	2.25	d
BAT 477 (nn)	3.63	bc	4.44	c	3.38	ab	3.75	abcd	3.00	cd
SEA5	4.38	b	8.75	a	3.88	ab	4.75	ab	3.00	cd
8-42-M-2	3.75	bc	3.13	cde	3.50	b	4.00	abcd	3.00	cd
DOR 364 (nod)	1.94	c	1.38	e	2.50	b	2.50	d	2.50	d
Mean	3.75		3.68		3.38		3.54		3.22	

‡ Indicates ozone count under irrigated conditions.

§ Indicates ozone count under rainfed conditions.

¶ Statistical significance of stress x genotype interaction (ozone count under irrigated and rainfed conditions).

\*\* , \* , +. Different letters indicates significant difference among means within a column at  $P \leq 0.01$ , 0.05 and 0.10, respectively, according to DMRT.

Table 5. Yield (kg ha<sup>-1</sup>) under irrigated and rainfed conditions, combined yield (kg ha<sup>-1</sup>), percent yield difference, 50 seed weight (g), seed per pod, number of pods harvested, pod weight (g), geometric mean (GM) (kg ha<sup>-1</sup>), drought susceptible index (DSI), and stress tolerance index (STI) of eight common bean (*Phaseolus vulgaris* L.) genotypes grown at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix Campus, U.S.V.I. 2000. N = 4 (rainfed and irrigated treatments) and 8.

Genotypes	Yield (Irrigated) (kg ha <sup>-1</sup> )	Yield (Rainfed) (kg ha <sup>-1</sup> )	†Comb. Yld. (kg ha <sup>-1</sup> )	% Yield Diff.	50 seed wt. (g)	Seed/pod	#Pods/m <sup>2</sup>	Pod wt./m <sup>2</sup> (g)	GM (kg ha <sup>-1</sup> )	DSI	STI
BAT 477 (nod)	1000ns‡	609 ns	804 ns	29	10.8 ns	3 ns	121 a**	39.74 ns	780	0.97	0.60
PR9603-22	940	735	838	22	10.8	6	111 ab	34.00	831	0.54	0.68
DOR 364 (nn)	1070	722	896	32	10.4	3	142 a	48.32	879	0.81	0.77
XAN 176	1291	440	865	66	10.8	3	133 a	41.16	754	1.64	0.56
BAT 477 (nn)	1070	560	815	48	9.9	3	105 ab	45.94	774	1.19	0.59
SEA5	877	743	810	15	11.5	3	114 ab	37.55	807	0.38	0.65
8-42-M-2	719	418	568	42	10.0	3	66 b	38.65	548	1.04	0.30
DOR 364 (nod)	1072	579	825	46	9.69	2	121 a	39.35	788	1.14	0.61
Mean	1005	601	803	---	10.5	3	114	40.58	---	---	---

† Indicates combined stress and non-stress yield.

‡ Indicates no significant difference among means within a column.

\*\* Different letters indicates significant difference among means within a column at P ≤ 0.01 according to DMRT.

DII value = 0.40

Table 6. Correlations of yield under stress, yield under non-stress, and combined yield for stress and non-stress treatments to geometric mean (GM), drought susceptibility index (DSI), and stress tolerance index (STI). Data from eight genotypes of common bean (*Phaseolus vulgaris* L.) grown at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix Campus, U.S.V.I. 2000.

	2000		
	GM	DSI	STI
Stress Yield	0.793*	-0.834**	0.821*
Non-stress Yield	0.510	0.626 <sup>+</sup>	0.462
Combined Yield	0.938***	-0.007	0.916**

<sup>+</sup>, <sup>\*</sup>, <sup>\*\*</sup>, <sup>\*\*\*</sup>. Indicates significance at  $P \leq 0.10, 0.05, 0.01$ , and  $0.001$ , respectively.



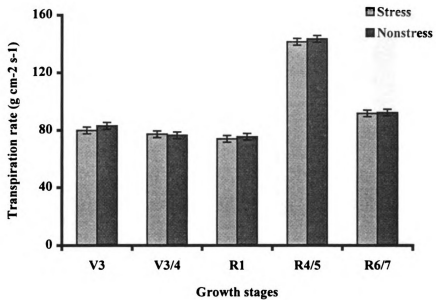


Figure 1. Transpiration rate (means  $\pm$  SE,  $n = 36$ ) of nine genotypes of common bean grown under irrigated and rainfed moisture conditions at the Agricultural Experiment Station at the University of the Virgin Islands, 1999. Vertical bars indicate standard error of the mean.

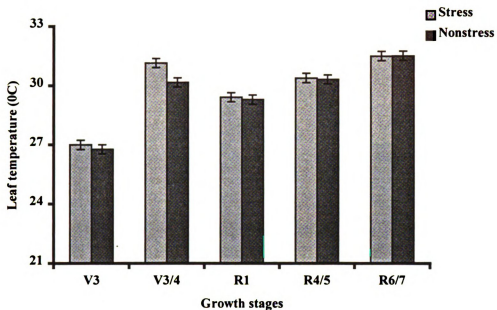


Figure 2. Leaf temperature (means  $\pm$  SE, n = 36) of nine common bean genotypes using a LiCor (LI 1600 Steady State) porometer grown under irrigated and rainfed moisture conditions at the Agricultural Experiment Station at the University of the Virgin Islands, 1999. Vertical bars indicate standard error of the mean.

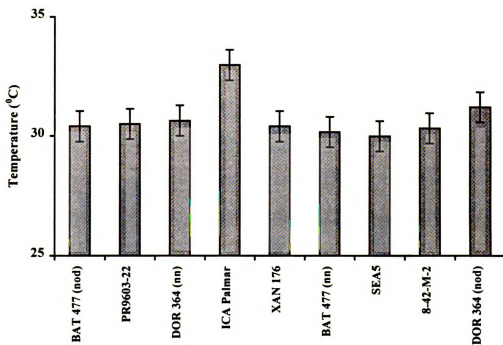


Figure 3. Leaf temperature (means  $\pm$  SE,  $n = 8$ ) of nine common bean genotypes taken with an infra-red thermometer at 57 DAP and grown under irrigated and rainfed moisture conditions at the Agricultural Experiment Station at the University of the Virgin Islands, 1999. Vertical bars indicate standard error of the mean at  $P \leq 0.10$ .

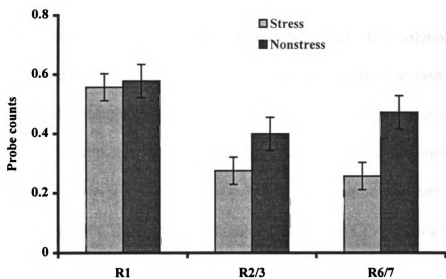


Figure 4. SENTRY probe counts (means  $\pm$  SE,  $n = 36$ ) of nine common bean genotypes taken at a depth of 30.5 cm and grown under irrigated and rainfed moisture conditions at the Agricultural Experiment Station at the University of the Virgin Islands, 1999. Vertical bars indicate standard error of the mean at R6/7 at  $P \leq 0.10$ .

## Chapter 2

### Root length, shoot weight, and root length density in common bean (*Phaseolus vulgaris* L.).

#### Abstract

One characteristic that may contribute to drought resistance in common bean is root mass. The objective of this study was to determine if drought resistant genotypes have differing root growth. Plants were grown in half-strength Hoagland's nutrient solution (control treatment) or half-strength Hoagland's nutrient solution +  $10^{-6}$  M abscisic acid (ABA treatment) in an environmentally controlled growth chamber. In another study, plants were grown in polyvinyl chloride (PVC) tubes in a glasshouse for 40 days. The ABA treatment increased total root length (TRL) and root length among root width classes. The susceptible check, 8-42-M-2 produced a greater portion of fine roots and a greater TRL than the resistant check, BAT 477 [nodulating (nod)]. In the PVC study, water deficit significantly reduced root width classes at all depths except at a depth of 30.6 - 45.7 cm and reduced TRL by approximately 75, 38, and 38% at depths of 0 - 15.2, 15.3 - 30.5, and 0 - 92 cm, respectively. Root length density was low ranging from 0.01 - 0.49 cm cm<sup>-3</sup>. Fine roots made the largest contribution to total root length in both stressed and nonstressed treatments. Growth pouch and PVC studies identified XAN 176, SEA5, and 8-42-M-2 as having high TRL, suggesting that growth pouches may provide a viable method for assessing root growth.

## **Introduction**

Identifying and understanding the mechanisms of drought tolerance in common bean (*Phaseolus vulgaris* L.) have been major goals of plant physiologists and breeders. Several mechanisms which permit common bean to achieve economic yields under drought environments have been proposed, including rooting depth (Sponchiado et al., 1989; White et al., 1990), ability to maintain stomatal opening at low levels of leaf water potential (Bates and Hall, 1981; Peng et al., 1991), high osmotic adjustment (Salih et al., 1999), stomatal conductance, and photosynthesis (Hamdani et al., 1991; Manthe, 1994). It is accepted that abscisic acid (ABA) acts as a stress hormone in plant systems, and the relationship between ABA levels and plant water status have been investigated extensively (Pierce and Raschke, 1981; Hartung and Davies, 1990). ABA may also play a role of importance in temperature stresses which affect plant water relations (Radin and Hendrix, 1986; Morgan, 1990).

Root characters are undoubtedly important in edaphic adaptation. Several researchers have shown that drought tolerance in common bean is related to depth of rooting (Sponchiado et al., 1989; White et al., 1990). Soil exploration by roots is associated with nutrient acquisition, especially in the case of immobile nutrients such as phosphorus (Lynch and van Beem, 1993; Yan et al., 1995a). Genetic differences have been reported in common bean for root biomass and root to shoot ratio (Fawole et al., 1982; Stofella et al., 1979a), and for root biomass distribution among distinct root types (Stofella et al., 1979b). In addition to parameters related to root size and growth, root architecture may be important for mining minerals, nutrients, and water from the soil

(Lynch and van Beem, 1993). Fitter (1991) developed topological indices to quantify root architecture in two-dimensions, ranging from a herringbone structure at one extreme to a highly branched, dichotomous structure at the other extreme (Fitter, 1991). Based on comparisons of ecologically distinct species, Fitter (1991) has proposed that root architecture may influence the efficiency of mineral, nutrient, and water acquisition from the soil.

Research on bean root growth has been conducted in hydroponics systems (Gabelman et al., 1986; Checkai et al., 1987), in field settings (Yan et al., 1995b), a spilt root system (Snapp and Lynch, 1996), and pots of different sizes (Lynch and van Beem, 1993; Yan et al., 1995a). Still, the understanding of the effects of moisture deficits on bean root growth remains at a rudimentary level. Yabba (1997) using a modification of the procedure used by Asady and Smucker (1989) observed root growth to a depth of 0.76 m in common bean grown for 40 days in polyvinyl chloride tubes.

Quantification of root growth and distribution is necessary to understand plant-soil interactions. However, root research has been hampered by inadequate, time-consuming methods (Persson, 1990). Advances in nondestructive methods of quantifying roots include nuclear magnetic resonance imaging (Rogers and Bottomley, 1987) and minirhizotron technologies (Taylor, 1987). Despite these efforts, there is a need for better knowledge and understanding of root growth and function as related to soil water status (Wraith and Wright, 1998). Hence, the objective of this study was to investigate the effects of water deficit and abscisic acid (ABA) on root length and root length density.

## Materials and methods

### *Growth chamber study:*

Two experiments were conducted in an environmentally controlled growth chamber to evaluate common bean seedling root growth: a control treatment in which plants were given only half-strength Hoagland's (Hoagland and Arnon, 1950) nutrient solution (control) and an abscisic acid (ABA) treatment consisting of half-strength Hoagland's nutrient solution +  $10^{-6}$ M ABA [*cis-trans*,  $\pm$  ABA, Sigma]. A 23/20°C day/night temperature and a 15 h photoperiod were used for both experiments.

Photosynthetically active radiation (PAR) was measured as  $876 \mu\text{mol m}^{-2}\text{s}^{-1}$  (control) and  $913 \mu\text{mol m}^{-2}\text{s}^{-1}$  (ABA) at the top of the of the plant canopy using a Decagon Sunfleck Ceptometer (Pullman, Wash.). Eight common bean genotypes with Type II and III growth habits were selected for study: BAT 477 (nod), PR9603-22, DOR 364 (nn) XAN 176, BAT 477 (nn), SEA5, 8-42-M-2, and DOR 364 (nod). The study utilized a randomized complete block design with four replications, days after transplant (14, 21, and 28 DAT) as the main plot, and genotype as subplot. Uniform sized seeds were selected and soaked in a  $1 \mu\text{mol CaSO}_4$  solution for one hour before germination. Seeds were germinated 5 days prior to the initiation of the experiment. Seedlings were transplanted, at one seed per pouch, to a specially designed growth pouch measuring 25.4 cm x 35.6 cm, an adaptation of a procedure used by McMichael et al. (1985), Merhaut et al. (1989), and Yabba (1997). All pouches were given 50 ml of half-strength Hoagland's nutrient solution (control treatment) or half-strength Hoagland's nutrient solution + ABA and adjusted to a pH of 6.14. Pouches were then stapled to a black cardboard and placed



upright in a specially designed holder with 2.54 cm between pouches. Seedlings were covered with a clear plastic covering for two days. Plants were given nine 50 ml applications of half-strength Hoagland's nutrient solution or half-strength Hoagland's nutrient solution + ABA from the sixth to the twenty-eighth DAT when the experiment was terminated. Plants were sampled at 14, 21, and 28 DAT. Fresh weights were taken for roots, stem, and leaves. Fresh roots were placed in a whirlpack bag and stored in 15% (v/v) methanol solution at 4°C. Leaves and stem were oven dried for 48 h at 60°C, weighed and discarded. Roots were prepared for root imaging according to the WinRhizo root imaging program (WinRhizo, Regent Instruments Inc.).

### ***Glasshouse study***

Plants were grown in polyvinyl chloride tubes (PVC) for 40 days in a glasshouse at Michigan State University, in East Lansing, MI. The temperature regime was 27°C ± 2°C and the light intensity was 1421  $\mu\text{E m}^{-2}\text{s}^{-1}$  with a 15 h photoperiod. Nine common bean genotypes with Type I, II, and III growth habits were grown: BAT 477 (nod), PR9603-22, DOR 364 (nn), ICA Palmer, XAN 176, BAT 477 (nn), SEA5, 8-42-M-2, and DOR 364 (nod). The experimental design was a split plot with water (stressed and non-stressed) as the main plot, genotypes as the subplot, and three replications. The PVC tubes were 0.92 m in length with a diameter of 30.5 cm. To determine root growth at different depths each PVC tube was cut into six 16.6 cm sections. The six individual sections were taped to produce one continuous tube. The bottom section was filled with silica sand. The remainder of the PVC tube was filled with a Metea loam (Loamy, mixed, mesic, Arenic Hapludalfs) that had been sieved through a 2 mm mesh wire and

packed to a bulk density of 1.37 g/cm<sup>3</sup>. Five seeds per PVC tube were planted on 7 August, 2000 and thinned to one plant per PVC tube at 14 days after planting (DAP). Stress was initiated at 14 DAP by cessation of water to plants in the stress treatment. Plants were given 18 L of water during the growing period (4 L before stress initiation and 14 L after stress initiation). Plants were sampled at R2 growth stage (40 DAP). Stem, leaf, and reproductive parts were weighed and dried at 60°C for 48 h, reweighed and then ground through a 1 mm screen Udy Cyclone Sample Mill (Udy Corporation, Fort Collins, CO.) for determination of total nitrogen. Roots were extracted from each section by sieving the soil through a 2 mm mesh wire. Fresh roots were placed in a whirlpack bag and stored in 15% (v/v) methanol solution at 4°C. Roots were prepared for root imaging according to the WinRhizo root imaging program (WinRhizo, Regent Instruments Inc.).

### ***Root Quantification***

The WinRhizo image analysis software was used to analyze the image root files acquired. Total area (image area), total volume, and average root diameter were calculated simultaneously by a procedure outlined by Tennant (1975). Roots were divided into 10 classes, based upon root diameter. The classes were: class 1 (0 - 0.5 mm), class 2 (0.51 - 1.0 mm), class 3 (1.01 - 1.5 mm), class 4 (1.51 - 2.0 mm), class 5 (2.01 - 2.5 mm), class 6 (2.51 - 3.0 mm), class 7 (3.01 - 3.5 mm), class 8 (3.51 - 4.0 mm), class 9 (4.01 - 4.5 mm), and class 10 (> 4.5 mm). In addition, root morphology measurements (length, volume, surface area) were calculated simultaneously with WinRhizo Regent's non-statistical method which estimates length distribution among specified root diameter ranges (WinRhizo User Manual, regent Instruments, Inc.). After scanning, roots were

oven dried at 60°C for 3 d, dry weights were recorded and then discarded. The MSTAT micro-computer statistical package (Michigan State University) for agricultural sciences was used for all data analysis.

## **Results and discussions**

### ***Root parameters: Growth chamber study.***

Root length was significantly higher in the ABA than in the control treatment for total root length (TRL) at 14 and 21 DAT and for all root classes except root class 9 at 21 DAT (Table 1). At 14 DAT, only root class 8 had a significant difference ( $P \leq 0.01$ ) between ABA and control treatments. At 28 DAT, significant differences existed between the two treatments for TRL and for root classes 2, 3, 7, and 9 (Table 1). ABA increased TRL by more than 50% at 21 and 28DAT. Results support previous work (Yabba, 1997) indicating ABA stimulation of root growth in common bean although that study concluded at 14 DAT.

There were no significant difference among genotypes at 14 DAT between treatments nor was there a genotype x experiment interaction (Table 2). At 21 DAT, the genotype XAN 176 had a significantly higher ( $P \leq 0.05$ ) TRL than all genotypes in the control treatment except SEA5 and the genotype SEA5 had a significantly higher TRL than all the genotypes in the ABA treatment except PR9603-22, DOR 364 (nn), and 8-42-M-2 (Table 2). At 21 DAT, the genotype SEA5 grown in the ABA treatment had a significantly higher ( $P \leq 0.10$ ) TRL than all genotypes except PR9603-22, DOR 364 (nn), and 8-42-M-2 in the ABA treatment (Table 2). There were no significant differences between BAT 477 (nod) and DOR 364 (nod) and their respective isolines at 21 DAT with

regard to TRL in ABA or control treatments. However, DOR 364 (nn) (ABA treatment) had a significantly ( $P \leq 0.10$ ) higher TRL than both DOR 364 (nn) and DOR 364 (nod) in the control treatment (Table 2).

At 28 DAT, there were no significant differences among the genotypes in the control treatment (Table 2). In the ABA treatment, the genotype 8-42-M-2 had a significantly higher ( $P \leq 0.01$ ) TRL than all genotypes except DOR 364 (nn) and a significantly higher ( $P \leq 0.01$ ) TRL than all genotypes in the control treatment (Table 2). There was no significant difference between BAT 477 (nod) and its isolate BAT 477 (nn) in the control or ABA treatments at 28 DAT but DOR 364 (nn) had a significantly higher TRL than its isolate DOR 364 (nod) in the ABA treatment and than DOR 364 (nn) in the control treatment at 28 DAT (Table 2).

The ABA treatment increased TRL on all sampling dates and increased the production of finer roots with greater than 99% of the roots occurring in root classes 1 and 2 (Table 1). This is significant because such an occurrence during a moisture deficit would increase the root absorptive surface area, thereby permitting the plant to obtain more soil moisture (Yabba, 1997). Roots generally explore and contact only 1 - 2% of the soil volume (Tesar, 1988), therefore an increase in root length increases the plant's ability to mine more water and nutrients. Since many root functions, such as water and ion uptake, are more closely related to root length than root volume (Waisel et al., 1996), the greater change in root length observed with the ABA treatment implies that plants growing in an ABA rich medium have a greater ability to obtain such resources. These results agree with other work indicating that ABA stimulates root growth (Yabba, 1997;

Sharp et al., 1993; Robertson et al., 1990; Creelman et al., 1990).

When ABA is applied to roots, the volume of water flow through the root is often increased, thereby increasing nutrient flow to the root (Cornish and Radin, 1990; Hegazi et al., 1999), hence resulting in more root growth. This is obviously an important attribute during water stress because it can improve the plant's water balance. This increased transport has been ascribed to either decreased hydraulic resistance in the roots (Glinka and Reinhold, 1971) or enhanced ion transport that increases the osmotic forces driving water flow through the root (Karmoker and van Steveninck, 1978).

At 14 DAT plants in both treatment may have been conducting very little photosynthesis and root growth may have been supported by photosynthates supplied by the cotyledons. If roots in both treatments were subjected to such a phenomenon, similar root growth among similar genotypes would be expected and the data at 14 DAT (Table 2) do reflect this.

From 21 DAT to 28 DAT plants may have started conducting photosynthesis and were producing more root hairs and more lateral roots. Waisel et al. (1996) has reported that root hairs and root laterals may be induced in an ABA liquid medium by increasing its oxygen content. Our study was conducted in an aqueous medium but we did not have an elevated oxygen content. Waisel et al. (1996) reported that one of the most obvious effects of ABA on *Brassicaceae* root growth was an increase in the number of lateral roots and an increase in both the number and length of the root hairs. However, it was not clear whether the enhancing effects of ABA on lateral root initiation and root hair formation resulted directly from inhibitory effects of ABA on the extension of the apical

root zone (Biddington and Dearman, 1982).

At 21 and 28 DAT, the ABA treatment of 8-42-M-2 produced greater root length than the ABA treatment for BAT 477 (nod), BAT 477 (nn), and DOR 364 (nod). These results are surprising since 8-42-M-2 is the drought susceptible check and BAT 477 (nod) the resistant check. We had postulated that drought resistant genotypes have a greater mass of fine roots than drought susceptible genotypes and that this might be one characteristic contributing to the drought resistance of BAT 477 (nod).

***Root parameters: Glasshouse.***

Water deficit significantly reduced common bean TRL in various root classes at all depths except at a depth of 30.6 - 45.7 cm (Table 3a and b). Total RL for the non-stress treatment at all depths was numerically higher than the stress treatment (Table 3a and b) and significantly higher in the top 30.5 cm (Table 3a). At all depths, the percent of roots less than or equal to 1.0 mm in diameter was 95% of the TRL for both stressed and nonstressed plants and at some depths it approached 100% of the TRL (Table 3a and b). There were no significant genotypic (stressed and nonstressed combined) differences at any depth except at 45.8 - 61 cm (Table 4). At the 45.8 - 61 cm depth, the genotype SEA5 had a significantly higher TRL ( $P \leq 0.10$ ) than the genotypes BAT 477 (nod), PR9603-22, DOR 364 (nn), and DOR 364 (nod) but not significantly higher than the other genotypes (Table 4). Each root width class was analyzed at each soil depth for genotypes (stress and nonstress RL combined), water, genotype x water interaction, stress RL, and nonstress RL (Tables 5 and 6). Among the genotypes, a cluster of significance

was observed for root width class 3 at a depth of 0 - 15.2, 15.3 - 30.5, 30.6 - 45.7, and 45.8 - 61 cm and another cluster at a depth of 45.8 - 61 cm (root width classes 1, 2, 3, 4, 5, 6, and 10) (Table 5). The data for significant differences among water treatments (Table 5) are presented in tables 3a and b. Significant genotype x water interaction was observed only at a depth of 0 - 15.2 cm ( $P \leq 0.10$ ) in root width class 4 and at a depth of 45.8 - 61 cm ( $P \leq 0.10$ ) in root class 5 (Table 5). In the stress treatment three clusters of significance were observed: TRL (root width classes 1, 2, 3, 4, 5, and 10), at depth 15.3 - 30.5 cm (root width classes 1, 2, 3, 4, and 10), and at depth 45.8 - 61 cm (root width classes 1, 2, 3, 4, 5, 6, and 10) (Table 6). In the nonstress treatment significant difference was only observed at a depth of 0 - 15.2 cm (root width classes 3 ( $P \leq 0.05$ ) and 4 ( $P \leq 0.05$ )) and depth 45.8 - 61 cm (root width classes 3 ( $P \leq 0.10$ ) and 4 ( $P \leq 0.10$ )) (Table 6). The data for all clusters are presented in tables 7 - 11. Thus, the genotypic differences largely result from genotypic differences under stress.

The first genotypic cluster consisted of root width classes 3, 4, and 10 at a depth of 1 - 15.3 cm and root width class 3 at depths 15.4 - 30.5 and 30.6 - 45.7 cm (Table 7) and the second cluster consisted of root width classes 1, 2, 3, 4, 5, 6, and 10 at depth 45.8 - 61 (Table 8). The genotype 8-42-M-2 had a significantly higher ( $P \leq 0.05$ ) class 3 RL than all genotypes except ICA Palmar and XAN 176 at depth 0 - 15.2 cm and the genotype XAN 176 had a significantly higher ( $P \leq 0.05$ ) class 4 RL than all other genotypes except ICA Palmar at depth 0 - 15.2 cm (Table 7). BAT 477 (nod) had a significantly greater ( $P \leq 0.10$ ) class 10 RL at depth 0 - 15.2 cm than PR9603-22, SEA5, 8-42-M-2, and DOR 364 (nod) (Table 7). The genotype XAN 176 had a significantly

greater ( $P \leq 0.10$ ) class 3 RL at depth 15.3 - 30.5 cm than all genotypes but not significantly greater than ICA Palmar, BAT 477 (nn), and 8-42-M-2. BAT 477 (nod) had a significantly higher ( $P \leq 0.10$ ) class 3 RL at depth 30.6 - 45.7 cm than PR9603-22, DOR 364 (nn), and DOR 364 (nod) (Table 7).

At depth 45.8 - 61 cm, the genotype XAN 176 had one of the highest root lengths at each of the classes (Table 8). XAN 176 was significantly higher than BAT 477 (nod), PR9603-22, and DOR 364 (nn) for all root width classes except root width class 6. The genotype 8-42-M-2 (susceptible check) was higher than BAT 477 (nod) only for root width class 1. The genotypes BAT 477 (nod), BAT 477 (nn), DOR 364 (nod), and DOR 364 (nn) did not differ significantly for root width classes 1 to 6 and 10 at depth 45.8 - 61 cm (Table 8).

In the first cluster among the stress treatment (Table 6) across all depths, the genotype SEA5 had one of the highest TRL's in all the root width classes analyzed, as did ICA Palmar, XAN 176, and 8-42-M-2 (Table 9). DOR 364 (nn) consistently had one of the lowest TRL's across root width classes. When comparing BAT 477 (nod), BAT 477 (nn), DOR 364 (nod), and DOR 364 (nn) the only difference was when TRL of BAT 477 (nn) exceeded that of DOR 364 (nn) for root width classes 3 and 10 (Table 9).

In the second cluster in the stress treatment which consisted of five root width classes at depth 15.3 - 30.5 cm, the genotype XAN 176 had one of the greatest RL's in root width classes 1 to 4 and 10, as did 8-42-M-2 (Table 10). In root width class 1, again DOR 364 (nn) had one of the lowest TRL's for root width classes 1 to 4 and 10 (Table 10).



In the third cluster which consisted of root width classes 1 to 6 and 10 at depth 45.8 - 61 cm, SEA5 had one of the greatest RL's in all the root width classes analyzed (Table 11). DOR 364 (nn) had one of the lowest RL's of all root width classes, although it did not differ significantly from BAT 477 (nod), BAT 477 (nn), and DOR 364 (nod) (Table 11).

These results indicate that root width classes 1, 2 and 3 (0 - 0.50, 0.51 - 1.0, and 1.01 - 1.50 mm, respectively) contributed the most to TRL among genotypes and treatments. It is also evident that some genotypes produce a greater portion of these root width classes than others indicating that fine root may be a meaningful trait that plant breeders can use in their efforts to breed for drought resistance in common bean.

Root dry weight (RDW), RL, root surface area (RSA), root volume (RV), and root length density (RLD) parameters were significantly lower in the stressed treatment at 0 - 15.2 and 15.3 - 30.5 cm depths (Table 12). Root diameter was only significant at 15.3 - 30.5 and 76.3 - 92 cm depths with the nonstress treatment having a higher root diameter (Table 12). Root length density, an index of water uptake capacity ranged from 0.01 - 0.49 cm cm<sup>-3</sup> and was significantly lower in the stress treatment at the 0 - 30.5 cm depth (Table 12). There was no significant difference in RLD in the 30.6 - 76.2 cm portion of the column, however RLD of the nonstressed treatment at all depths was numerically higher than the stressed treatment (Table 12). There were no significant differences between stress and nonstress treatment among any of the root parameters at a depth of 30.6 to 76.2 cm, however RDW and RD was significantly higher in the nonstress treatments at depth 76.3 to 92 cm (Table 12).

There were significant genotypic differences for combined stress and nonstress RLD only at depths 45.8 - 61 cm (combined stress and nonstress RLD) and 15.3 - 30.5 cm (stress treatment) (Table 13). At depth 15.3 - 30.5 cm in the stress treatment, the genotype XAN 176 had a significantly higher ( $P \leq 0.01$ ) RLD than all the genotypes except BAT 477 (nn) and 8-42-M-2. At depth 45.8 - 61 cm, the combined stress and nonstress RLD of XAN 176 was significantly higher ( $P \leq 0.10$ ) than BAT 477 (nod), DOR 364 (nn), and DOR 364 (nod) but not significantly higher than the other genotypes (Table 13). There were no significant difference in RLD between the two non-nodulating line (BAT 477 (nod) and DOR 364 (nod)) and their respective isolines at any of the depths (Table 13). There was no significant difference between the resistant check (BAT 477 (nod)) and the susceptible check (8-42-M-2) at depth 15.3 - 30.5 cm, of the stress treatment, but there was at depth 45.8 - 61 cm for combined RLD with 8-42-M-2 having a higher RLD (Table 13).

Results indicated that fine roots ( $\leq 1.0$  mm diameter) made the largest contribution to total root length in both stressed and nonstressed treatments. The data suggest that water absorption may be more associated with fine than large roots. Water stress reduced TRL by approximately 75, 38, and 38%, respectively, at depths of 0 - 15, 15.1 - 30.5, and 0 - 92 cm.. Results support work by others indicating that the distribution of roots in a soil profile is largely a function of depth (Box, 1996) and work indicating that rooting depth and root system development are closely related to soil moisture content (Asady and Smucker, 1989; Waisel et al., 1996; Manschadi et al., 1998). In a dry soil, root distribution and downward penetration of roots are restricted due to an

increase in soil strength (Gerard et al., 1982; Jones et al., 1991). If water stress is moderate to severe, downward root growth will be slowed (Ehlers et al., 1983; Bennie and Botha, 1986; Manschadi et al., 1998) resulting in a shallower rooting depth. If water stress persists long enough to prevent root growth from extending into the deeper soil layers, the total root system will be restricted to the upper part of the profile (Chaudhary et al., 1985). My results showed that stress had an effect on the rooting depth (lessening RL to the lower soil profile) and root length (decreasing RL among all root width classes and at all soil depths) and are consistent with similar findings in faba-bean (*Vicia faba* L.) (Manschadi et al., 1998; Heeraman and Juma, 1993) and barley (*Hordeum vulgare* L.) (Manschadi et al., 1998).

Since root size and morphology are important in the efficient uptake of nutrients and minerals (Sullivan et al., 2000), detecting differences in root growth patterns and length between common bean genotypes may offer unique selection criteria for drought tolerance. The genotype SEA5 produced the greatest combined RL at a depth of 45.8 - 61 cm (Table 4), the greatest TRL in all the root width classes in the stress treatment at 15.3 - 30.5 cm (Table 9), and the greatest RL among all root width classes analyzed at a depth of 45.8 - 61 cm in the stress treatment (Table 11). Under moisture stress at depths of 15.5 - 30.5 cm and when treatments were combined at depths up to 61 cm, the genotype XAN 176 had the greatest RL in most of the root width classes (Tables 7 and 8). Results suggest that SEA5 and XAN 176 allocated more of their photosynthates into root production under water stress, maybe at the expense of aboveground production. In the water stress treatment, the resistant check BAT 477 (nod) produced a RL that was

consistently lower in all root width classes examined than both SEA5 and XAN 176 suggesting that maybe one of the mechanisms for the designation of BAT 477 (nod) as being drought resistant is in its ability to allocate more photosynthates into shoot production than root production under periods of water stress. How this relates to the efficiency of this genotype still needs to be investigated. The data also suggests that most of the genotypes invested a lot of resources into producing an increased quantity of finer roots in the stress treatment, supporting the importance of small or fine roots in relation to plant stress for the mining of water (Marschner, 1997; Manschadi et al., 1998).

In my study, RLD was low (Tables 12 and 13) compared to values of 0.5 - 2.0 cm cm<sup>-3</sup> reported by de Willigen and van Noordwijk (1987) for common bean in the 0 - 30 cm depth and 1.55 - 3.1 cm cm<sup>-3</sup> reported by Heeraman and Juma (1993) for faba-bean in the 0 - 30 cm depth. The low values obtained here could be a reflection of the method used in obtaining root samples. Heeraman and Juma's (1993) results were obtained using minirhizotron, core samples, and the monolith method and de Willigen and van Noordwijk (1987) results reported using a minirhizotron. Our results were obtained by sieving soil samples through a 2 mm mesh screen which only has the potential of collecting an average of 55% of the root weight and only 10% of the plant TRL (Amato and Pardo, 1994). The loss of fine roots from a 0.5 mm<sup>2</sup> mesh sieve has been reported to vary according to root integrity related to plant age (Boehm, 1979). Similarly, Amato and Pardo (1994) found that different methods of sample preparation could affect root integrity and therefore change the amount of fine roots retained by coarse sieves.

The high RLD exhibited by the genotype XAN 176 shows this genotype's

potential for producing a deep and expansive root system even in water stress environments which is also reflected in the production of finer roots deeper into the soil profile (Tables 7 and 8). High RLD in the surface layer is a favorable characteristic of crops in semiarid areas to allow for ready absorption of water after rain and to minimize evaporation (Lampurlanes et al., 2001). Root growth deep in the soil profile allows a crop to explore a greater volume of soil and consequently to access more water (Box, 1996). Root LD normally increases from date of planting of annuals and decreases with soil depth and environmental root stress (Box, 1996). Results from this study showed RLD started to decrease at a depth of 45 cm (Table 12) which is in agreement with other studies reported in the literature (de Willigen and van Noordwijk, 1987; Heeraman and Juma, 1993).

#### ***Shoot and root dry weight and R/S***

Abscisic acid increased root and shoot dry weights at 21 DAT and increased root/shoot ratio (R/S) at 21 and 28 DAT (Table 14). The ABA treatment increased both shoot and root dry weights and R/S over the control treatment and ABA treatment increased shoot and root growth by 47 and 49%, respectively, at 14 DAT and 21 DAT while shoot and root increased by 31 and 37%, respectively, in the control treatment. However, between 21 DAT and 28 DAT, the control treatment had a higher increase in shoot and root dry weight (Table 14).

#### ***Control genotypic response:***

In the control treatment at 14 DAT, XAN 176 had a higher shoot dry weight than BAT 477 (nod), DOR 364 (nn), BAT 477 (nn), and DOR 364 (nod), but a root dry weight

only higher than DOR 364 (nn). The only difference in R/S ratio was a significantly higher ratio in SEA5 than in PR9603-22 (Table 15).

At 21 DAT, the genotype XAN 176 recorded the highest shoot and root dry weight (Table 15). XAN 176 had a significantly higher shoot weight ( $P \leq 0.10$ ) than all the genotypes except PR9603-22 and a higher root dry weight ( $P \leq 0.01$ ) than all other genotypes except PR9603-22 (Table 15). Root/shoot ratio ranged from 0.25 to 0.53, with the genotype SEA5 having a significantly greater ( $P \leq 0.01$ ) R/S than all other genotypes.

At 28 DAT, there was no significant difference observed among the genotypes for root dry weight and R/S ratio (Table 15). The genotype XAN 176 had a significantly higher ( $P \leq 0.05$ ) shoot weight than all the genotypes except BAT 477 (nod), PR9603-22, BAT 477 (nn), and DOR 364 (nod) (Table 15).

***ABA genotypic response:***

In the ABA treatment, at 14 DAT, only R/S ratio was statistically significant with the genotypes 8-42-M-2 and DOR 364 (nod) having a significantly greater ( $P \leq 0.01$ ) R/S than the genotypes PR9603-22, DOR 364 (nn), and XAN 176, but not significantly higher than the other genotypes (table 16).

At 21 DAT, there was no significant difference among the genotypes for shoot dry weight (Table 16). The genotype SEA5 had a significantly higher ( $P \leq 0.05$ ) root dry weight than BAT 477 (nod), DOR 364 (nn), BAT 477 (nod), and DOR 364 (nod) and a significantly higher ( $P \leq 0.05$ ) R/S ratio than PR9603-22, DOR 364 (nn), and BAT 477 (nn) (Table 16).

At 28 DAT, DOR 364 (nn) had a significantly higher ( $P \leq 0.01$ ) shoot dry weight

than BAT 477 (nod), PR9603-22, BAT 477 (nn), and DOR 364 (nod) (Table 16). The genotype 8-42-M-2 had a significantly higher ( $P \leq 0.01$ ) root dry weight than all the other genotypes except DOR 364 (nn), XAN 176, and SEA5 (Table 16). Root/shoot ratio ranged from 0.30 to 0.52 with the genotypes SEA5 and 8-42-M-2 having a significantly higher ( $P \leq 0.10$ ) R/S ratio than PR9603-22 and DOR 364 (nn) (Table 16).

The genotype XAN 176 consistently had one of highest shoot and root dry weights in the control treatment. The genotype SEA5 had one of the highest root dry weights and R/S ratios at 14 and 21 DAT. In the ABA treatment, there was no consistent performance among genotypes with regard to shoot dry weight. The top performers for root dry weight and R/S ratio were SEA5 and 8-42-M-2, both type III beans. In the ABA treatment at 14 and 28 DAT, DOR 364 (nod) had a higher R/S ratio than DOR 364 (nn), due to the lower shoot dry weight of the nodulating line.

***PVC genotypic response:***

Water deficit significantly decreased the accumulation of dry matter in leaves ( $P \leq 0.001$ ), stems ( $P \leq 0.01$ ), reproductive parts ( $P \leq 0.05$ ), shoots ( $P \leq 0.001$ ), and roots ( $P \leq 0.05$ ) in the PVC treatment and increased R/S ratio ( $P \leq 0.10$ ) (Table 17). There were no significant genotypic differences for leaf, stem, and shoot dry weight and R/S ratio for combined data (stress and nonstress combined) but there were significant differences among the genotype for reproductive parts ( $P \leq 0.001$ ) and root dry weight ( $P \leq 0.10$ ) (Table 18). The genotype SEA5 had a greater reproductive dry weight than all the genotypes except BAT 477 (nod) and PR9603-22. SEA5 and XAN 176 had a higher root dry weight than DOR 364 (nn) and DOR 364 (nod) somewhat similar to SEA5's relative

performance to DOR 364 (nod) and DOR 364 (nn) in the growth pouch studies. BAT 477 (nod) had a lower root dry weight under stress than did BAT 477 (nn). Results suggest that the greater drought resistance of BAT 477 (nod) in comparison to 8-42-M-2 is not due to decreased root length of 8-42-M-2.

When analyzed by water treatment there was significant genotypic difference for stem, reproductive parts, and root dry weight in the stress treatment and reproductive parts and R/S ratio in the nonstress treatment (Table 19). In the stress treatment, the genotype PR9603-22 had a significantly higher ( $P \leq 0.10$ ) stem dry weight than BAT 477 (nod), DOR 364 (nn), ICA Palmar, and SEA5 (Table 19). The genotype SEA5 had a significantly higher ( $P \leq 0.01$ ) reproductive dry weight than all the genotypes except PR9603-22 and a significantly greater ( $P \leq 0.01$ ) root dry weight than all the genotypes except BAT 477 (nn) (Table 19). In the nonstress treatment, again SEA5 had a significantly greater ( $P \leq 0.05$ ) reproductive dry weight than all the genotypes except BAT 477 (nod), PR9603-22, and BAT 477 (nn) (Table 19). The genotype XAN 176 had the highest R/S ratio ( $P \leq 0.10$ ) among the genotypes in the nonstress treatment (Table 19).

The data show that some genotypes allocated a higher proportion of biomass to roots than others and support the concept that root growth in dry soils is usually reduced less than shoot growth, leading to a typical increase in R/S ratio in response to drought stress (Waisel et al., 1996).

The performance of XAN 176 and SEA5 is quite intriguing. Both genotypes demonstrated that more photosynthates were allocated to root production during water



stress, however, the yield reported for both of these genotypes (Table 2, chapter 1) is quite different. The genotype XAN 176 produced yields in excess of 1000 kg ha<sup>-1</sup> in both irrigated and rainfed conditions compared to SEA5 which produced approximately 600 kg ha<sup>-1</sup> under both irrigated and rainfed conditions. The low yield of SEA5 is probably explained by the fact that SEA5 experienced heavy infestation by *Cercospora*, common bacterial blight infection, and ozone injury, all of which undoubtedly had an impact on its yield. It could also be speculated from this study that XAN 176 allocated more photosynthates into seed production instead of shoot production than SEA5. Generally, BAT 477 (nn) outperformed BAT 477 (nod) in PVC and growth pouch studies with regard to shoot and root growth and RLD whenever significant differences or tendencies occurred, while DOR 364 (nod) usually outperformed DOR 364 (nn).

Water stress increases root-shoot ratio and the ratio of root to shoot growth varies widely between plant species and is strongly modified by external factors (Marschner, 1997). In annual species competition for photosynthates between shoot and roots becomes the dominant factor during reproductive growth in limiting root growth and activity. The proportion of photosynthates allocated belowground and used for fine root production can be up to 44% in a tropical broadleaf stand (Cuevas et al., 1991). For example, in maize seedlings without drought stress the R/S ratio is 1.45 compared with 5.79 under drought stress (Sharp et al., 1993). The results gathered from this study support previous reports (El Nadi et al., 1969; Muchow, 1985; Haggani and Pandey, 1993; Manthe, 1994) for beans grown under drought environment.

### **Conclusion**

The ABA treatment increased TRL on all sampling dates over the control treatment and increased the production of finer roots at 21 DAT. The susceptible check, 8-42-M-2, produced significantly greater RL (5.47 m) than the resistant check BAT 477 (nod) (2.42 m), and the ABA treatment increased 8-42-M-2 TRL two-fold between each harvest date. Fine roots ( $\leq 1.0$  mm diameter) made the largest contribution to total root length in both stressed and nonstressed treatments, suggesting that water absorption may be more associated with fine than large roots. Water stress treatment reduced TRL by approximately 75, 38, and 38%, respectively, at depths of 0 - 15, 15.1 - 30.5, and 0 - 92 cm.. Root length density was low, probably reflecting the method used to obtain samples. The genotypes XAN 176 and SEA5 allocated a higher proportion of biomass to roots than did other genotypes. Results suggest that some genotypes produce a greater portion of roots in root width classes 1, 2, and 3, indicating that fine roots may be a meaningful trait that plant breeders can use in their efforts to breed for drought resistance in common bean.

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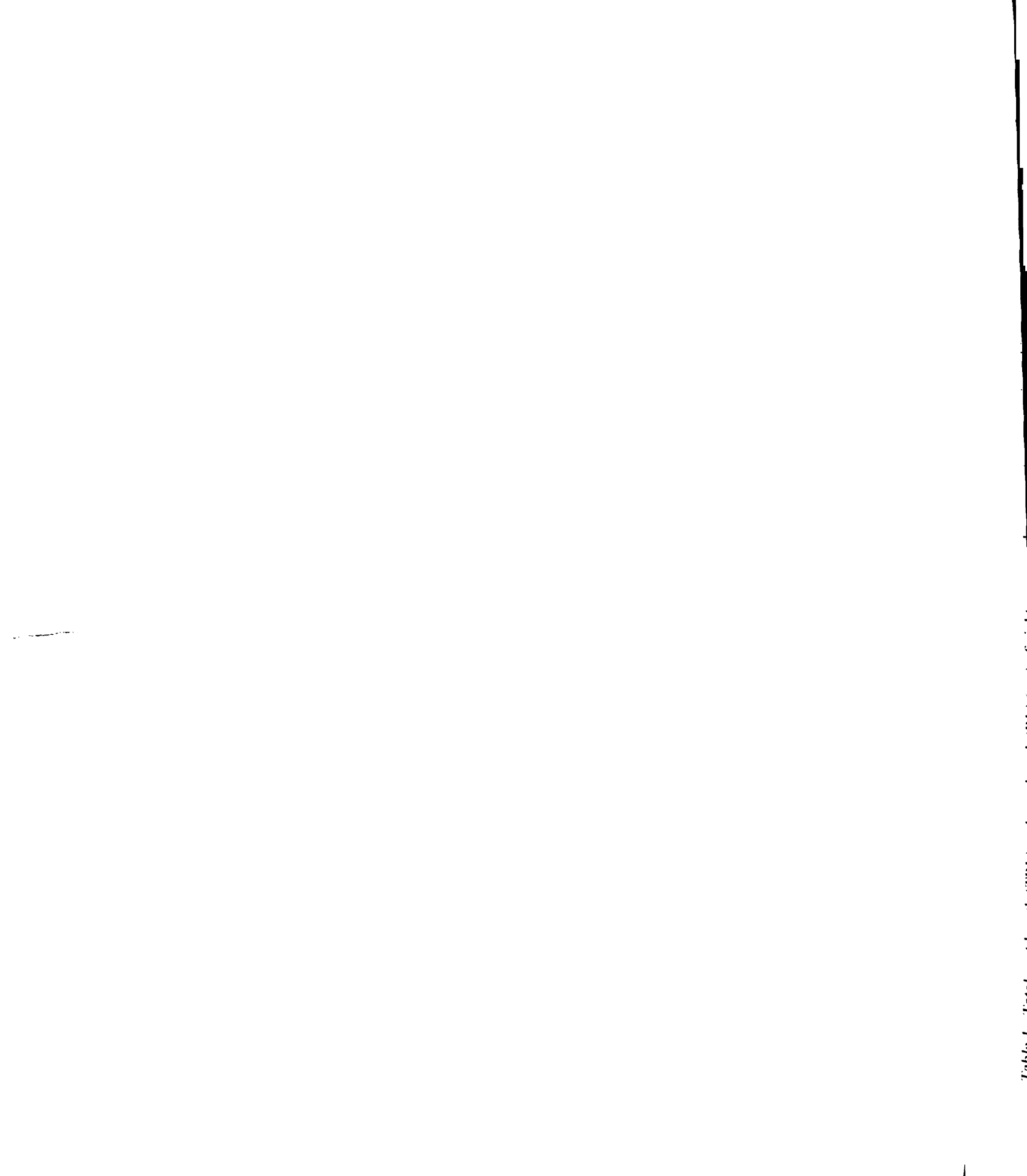


Table 1. Total root length (TRL) and root length (RL) (cm) of eight common bean genotypes germinated in a germination chamber for 4 d at 25°C and transplanted to an environmentally controlled growth chamber for 28 d at 23/20°C day/night temperatures and a 15 h photoperiod and grown under control conditions in a half-strength Hoagland's solution or in 10<sup>-6</sup> M ABA solution. Roots were harvested at 14, 21, and 28 days after transplanting (DAT) and divided into 10 classes based upon root diameter. n = 32.

	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7	Class 8	Class 9	Class 10	
TRL	0-0.5mm	0.51-1.0mm	1.01-1.5mm	1.51-2.0mm	2.01-2.5mm	2.51-3.0mm	3.01-3.5mm	3.51-4.0mm	4.01-4.5mm	>4.5mm	
<b>14 DAT</b>											
ABA	2017 ns§	1437 ns	533 ns	35 ns	6 ns	1.3 ns	0.29 ns	0.16 ns	0.09**	0.05 ns	3.6 ns
Control	1899	1402	453	32	5	1.0	0.20	0.10	0.05	0.04	3.5
<b>21 DAT</b>											
ABA	3999**	2880*	1039***	58*	10**	2.4*	0.70*	0.25*	0.12 <sup>+</sup>	0.09 ns	7.8**
Control	2511	1884	550	40	6	1.4	0.44	0.10	0.05	0.07	4.2
<b>28 DAT</b>											
ABA	5776 <sup>+</sup>	4336 ns	1313*	90 <sup>+</sup>	18 ns	4.8 ns	1.8 ns	0.66 <sup>+</sup>	0.32 ns	0.10*	11 ns
Control	3760	2835	809	59	12	3.5	1.2	0.37	0.13	0.03	8

§ Indicates no significant difference among means within a column.

\*\*\*, \*\*, \*, + Indicates significant difference among means within a column at P ≤ 0.001, 0.01, 0.05, and 0.10, respectively, according to DMRT.



Table 2. TRL (m) at each harvest date for eight genotypes of common bean (*Phaseolus vulgaris* L.) plants germinated in a germination chamber for 4 d at 25°C and transplanted to an environmentally controlled growth chamber for 28 d at 23/20°C day/night temperatures and a 15 h photoperiod and grown under control conditions in a half-strength Hoagland's solution or in 10<sup>-6</sup> M ABA solution. N = 4.

Genotypes	14 DAT		21 DAT		28 DAT	
	Control	ABA	Control	ABA	Control	ABA
BAT 477 (nod)	‡ns 17.7 ns§	ns 15.1 ns	b* 20.6 e+	c+ 31.4 cde+	ns 34.2 def**	d** 39.3 cdef**
PR9603-22	17.5	17.0	b 25.1 de	ab 48.6 ab	33.2 def	cd 43.0 cdef
DOR 364 (nn)	15.3	20.2	b 22.2 e	abc 41.0 abc	32.2 def	ab 76.0 ab
XAN 176	23.5	18.5	a 37.4 bcd	bc 33.6 cde	43.0 cdef	bcd 60.4 bcd
BAT 477 (nn)	17.5	21.1	b 22.4 e	c 32.6 cde	45.3 cdef	cd 49.4 cdef
SEAS	21.4	23.3	ab 31.6 cde	a 51.2 a	31.1 ef	bc 64.6 abc
8-42-M-2	21.7	27.2	b 21.9 e	ab 48.8 ab	26.0 f	a 88.2 a
DOR 364 (nod)	17.3	19.1	b 19.7 e	c 32.8 cde	55.8 bcde	cd 41.3 cdef
Mean	18.99	20.17	25.11	40.00	37.6	57.75

‡ Indicates statistical analysis among means within a column, ns indicates no significant difference and different letters indicate significant difference according to DMRT.

§ Indicates statistical analysis between treatments at 14, 21, or 28 DAT, ns indicates no significant difference and different letters indicate significant difference according to DMRT.

\*\*\*, \*\*, \*, + Indicates significant difference among means within a column at P ≤ 0.001, 0.01, 0.05, and 0.10, respectively, according to DMRT.



Table 3a. Root length (RL) (cm) of nine different root width classes of common bean grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions. n = 27.

		Root width classes								
		Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7	Class 8	Class 10
		0-0.5 mm	0.51 - 1.0 mm	1.01 - 1.5 mm	1.51 - 2.0 mm	2.01 - 2.5 mm	2.51 - 3.0 mm	3.01 - 3.5 mm	3.51 - 4.0 mm	> 4.5 mm
		<b>Root length (cm)</b>								
		<u>0 - 15.2 cm depth</u>								
Stress	700**	152**	14**	3**	1 ns†	0.37 ns	0.08 ns	0.02 ns	2**	
Nonstress	2741	582	73	13	3	0.66	0.13	0.02	9	
		<u>15.3 - 30.5 cm depth</u>								
Stress	2485*	414*	29*	3.5 ns	0.5 ns	0.05+	0.01 ns	nd	7*	
Nonstress	3991	850	56	7.1	0.9	0.20	0.03	nd	12	
		<u>30.6 - 45.7 cm depth</u>								
Stress	3367 ns	717 ns	36 ns	3.5 ns	0.44 ns	0.07 ns	0.01 ns	nd	10 ns	
Nonstress	4797	1116	68	7.2	1.0	0.17	0.02	nd	15	

Table 3b.

		Root width classes									
		Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7	Class 8	Class 10	
		0-0.5 mm	0.51 - 1.0 mm	1.01 - 1.5 mm	1.51 - 2.0 mm	2.01 - 2.5 mm	2.51 - 3.0 mm	3.01 - 3.5 mm	3.51 - 4.0 mm	> 4.5 mm	
	Root length (cm)										
	<u>45.8 - 61 cm depth</u>										
Stress		2180 ns	700 ns	29+	3.2+	0.41+	0.04+	0.004+	nd§	8 ns	
Nonstress		3270	948	55	5.9	0.69	0.07	0.01	nd	10	
	<u>61.1 - 76.2 cm depth</u>										
Stress		1396 ns	435 ns	18*	2*	0.21+	0.01 ns	nd	nd	4.2 ns	
Nonstress		1638	641	40	4	0.55	0.10	nd	nd	5.5	
	<u>76.3 - 92 cm depth</u>										
Stress		75 ns	51 ns	3.4+	0.42+	0.09+	0.01 ns	nd	nd	0.3 ns	
Nonstress		88	69	7.6	0.84	0.13	0.03	nd	nd	0.3	
	TRL										
Stress		10202+	2470+	816 ns	15.7*	2.71 ns	0.60 ns	0.11 ns	0.02+	31.01+	
Nonstress		16520	4205	1349	37.7	6.05	1.23	0.21	0.04	51.51	

\* , + Indicates significant difference among means within a column at P ≤ 0.01, 0.05, and 0.10, respectively, according to DMRT.

‡ Indicates no significant difference among means within a column.

§ No roots detected for this root diameter and rooting depth.

Table 4. Total root length (m) (TRL) at 15.24 cm depth increments for nine common bean genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions. n = 6.

Genotypes	TRL (m)	‡1 - 15.2 cm	15.3 - 30.5 cm	30.6 - 45.7 cm	45.8 - 61 cm	61.1 - 76.2 cm	76.3 - 92 cm
BAT 477 (nod)	169.92 ns§	25.2 ns	40.8.3 ns	66.2 ns	21.0 c+	15.3 ns	1.40 ns
PR9603-22	162.92	18.3	39.2	47.2	32.3 abc	24.6	1.38
DOR 364 (nn)	119.00	24.2	31.4	32.5	21.6 c	9.33	0.00
ICA Palmar	185.05	18.8	41.0	54.6	35.6 abc	32.8	2.15
XAN 176	194.72	20.7	47.5	53.5	58.4 a	14.0	0.70
BAT 477 (nn)	192.91	27.3	48.0	65.6	37.0 abc	13.9	1.22
SEA5	190.62	14.0	28.1	50.6	49.5 ab	44.7	3.61
8-42-M-2	191.09	20.5	40.0	45.6	56.9 ab	26.6	1.63
DOR 364 (nod)	134.71	20.3	37.8	38.1	30.1 bc	7.18	1.23
Mean	171.22	21.0	39.3	50.4	38.0	20.9	1.48

‡ Indicates depths.

§ Indicates no significant difference among means within a column.

+ Indicates significant difference among means within a column at  $P \leq 0.10$  according to DMRT.



Table 5. Statistical significance from ANOVA for genotypes, water, and genotype x water interaction for all root width classes and rooting depths of nine common bean genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions. n = 6 (genotypes), 27 (water), and 3 (genotype x water).

Classes	Genotypes						Water						Genotype x water					
	A§	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
†1	ns¶	ns	ns	★	ns	ns	★★	★	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	★★	ns	ns	★★	★	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	★	†	†	★★	ns	ns	★★	★	ns	†	†	†	ns	ns	ns	ns	ns	ns
4	★	ns	ns	★★	ns	ns	★★	ns	ns	†	†	†	†	ns	ns	ns	ns	ns
5	ns	ns	ns	★	ns	ns	ns	ns	ns	†	†	†	ns	ns	ns	†	ns	ns
6	ns	ns	ns	★	ns	ns	ns	†	ns	†	ns	ns	ns	ns	ns	ns	ns	ns
7	ns	ns	ns	ns	---	---	ns	ns	ns	†	---	---	ns	ns	ns	ns	---	---
8	ns	---	---	---	---	---	ns	---	---	---	---	---	ns	---	---	---	---	---
9	ns	---	---	---	---	---	ns	---	---	---	---	---	ns	---	---	---	---	---
10	†	ns	ns	†	ns	ns	★★	★	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

† Class 1 - 10 indicate respective root width classes beginning at 0 - 0.5, 0.51 - 1.0, 1.01 - 1.5, 1.51 - 2.0, 2.01 - 2.5, 2.51 - 3.0, 3.01 - 3.5, 3.51 - 4.0, 4.01 - 4.5, and greater than 4.5 millimeters.

§ Indicates Depth "A" = 1-15.2 cm, "B" = 15.3-30.5 cm, "C" = 30.6-45.7 cm, "D" = 45.8-61 cm, "E" = 61.1-76.2 cm, and "F" = 76.3-92 cm.

¶ Indicates no significant difference among means within a column.

★★, ★, +. Indicates significance at 0.01, 0.05, and 0.10, respectively, according to DMRT.

Table 6. Statistical analysis from ANOVA for genotypic response of nine common bean genotypes for all root width classes and rooting depths when grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions. n = 3.

Classes	Stress										Nonstress											
	TRL	§“A”	“B”	“C”	“D”	“E”	“F”	TRL	“A”	“B”	“C”	“D”	“E”	“F”	TRL	“A”	“B”	“C”	“D”	“E”	“F”	
1	★	ns¶	★★	ns	†	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	★★	ns	★★	ns	★	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	†	ns	★★	†	★★	ns	ns	ns	★	ns	ns	†	ns	ns	ns	ns	ns	ns	†	ns	ns	ns
4	★	ns	†	ns	★★	ns	ns	ns	★	ns	ns	†	ns	ns	ns	ns	ns	ns	†	ns	ns	ns
5	†	ns	ns	ns	★	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	ns	ns	ns	ns	★★	ns	ns	ns	★★	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
7	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
8	ns	ns	---	---	---	---	---	ns	---	---	---	---	---	---	---	---	---	---	---	---	---	---
9	ns	ns	---	---	---	---	---	ns	---	---	---	---	---	---	---	---	---	---	---	---	---	---
10	★★	ns	★	ns	†	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

† Class 1 - 10 indicate respective root width classes beginning at 0 - 0.5, 0.51 - 1.0, 1.01 - 1.5, 1.51 - 2.0, 2.01 - 2.5, 2.51 - 3.0, 3.01 - 3.5, 3.51 - 4.0, 4.01 - 4.5, and greater than 4.5 millimeters.

★★, \*, †. Indicates significance at 0.01, 0.05, and 0.10, respectively, according to DMRT.

§ Indicates Depth “A” = 1-15.2 cm, “B” = 15.3-30.5 cm, “C” = 30.6-45.7 cm, “D” = 45.8-61 cm, “E” = 61.1-76.2 cm, and “F” = 76.3-92 cm.

¶ Indicates no significant difference among means within a column.

Table 7. Combined root length (cm) from stressed and nonstressed moisture conditions of nine common bean (*Phaseolus vulgaris* L.) genotypes of root width class 3, 4, and 10 at depth 0 - 15 cm depth, root width class 3 at 15.3 - 30.5 cm, and 30.6 - 45.7 cm depths when grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod in stress and non-stress conditions.

n = 6.

Genotypes	0 -0.5 mm	1.51 -2.0 mm	> 4.5 mm	1.01 - 1.5	1.01 - 1.5
	Class 1	Class4 "A"	Class10 "A"	Class3 "B"	Class3 "C"
	0 - 15.2 cm	0 - 15.2 cm	0 - 15.2 cm	15.3 - 30.5 cm	30.6 - 45.7 cm
BAT 477 (nod)	41.26 b*	6.10 b*	8.24 a+	38.11 bcd+	76.87 a+
PR9603-22	42.78 b	5.56 b	4.18 bc	37.53 bcd	35.10 bcd
DOR 364 (nn)	36.59 b	7.76 b	6.00 abc	29.45 d	29.57 d
ICA Palmar	54.09 ab	9.83 ab	5.01 abc	55.07 ab	62.18 abc
XAN 176	59.06 ab	14.5 a	5.93 abc	59.32 a	63.27 abc
BAT 477 (nn)	42.14 b	6.15 b	7.10 ab	43.52 abcd	64.83 ab
SEA5	32.93 b	4.73 b	3.45 c	35.87 bcd	54.00 abcd
8-42-M-2	74.48 a	8.86 b	4.77 bc	50.09 abc	47.92 abcd
DOR 364 (nod)	40.30 b	7.43 b	4.72 bc	34.30 cd	32.55 cd
Mean	47.07	7.80	5.50	42.58	51.81

\*\* , \* , +. Indicates significance at 0.01, 0.05, and 0.10, respectively, according to DMRT.

**Table 8.** Combined root length (cm) of root width classes 1, 2, 3, 4, 5, 6, and 10 at 45.8 - 61 cm depth, from stressed and nonstressed moisture conditions of nine common bean (*Phaseolus vulgaris* L.) genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod. n = 6.

Genotypes	Class 1 0 - 0.5 mm	Class 2 0.51 - 1.0 mm	Class 3 1.01 - 1.5 mm	Class 4 1.51 - 2.0 mm	Class 5 2.01 - 2.5 mm	Class 6 2.51 - 3.0 mm	Class 10 > 4.5 mm
BAT 477 (nod)	1152 c+	458.4 cd*	28.30 b**	2.90 bc**	0.398 bc*	0.063 bc*	4.66 c+
PR9603-22	1931 bc	759.5 bcd	26.07 b	2.54 bc	0.255 c	0.019 c	7.04 bc
DOR 364 (nn)	1374 c	425.1 d	19.43 b	2.30 bc	0.355 bc	0.021 c	4.73 c
ICA Palmar	2356 abc	652.9 bcd	42.18 ab	3.75 bc	0.528 abc	0.042 bc	9.07 abc
XAN 176	4264 a	1467 a	81.43 a	9.91 a	1.183 a	0.128 ab	14.2 a
BAT 477 (nn)	2888 abc	753.1 bcd	45.41 ab	4.00 abc	0.373 bc	0.018 c	8.83 abc
SEA5	3702 ab	1164 ab	63.27 ab	8.14 ab	0.972 ab	0.161 a	12.5 ab
8-42-M-2	4481 a	1130 abc	56.44 ab	5.83 abc	0.596 abc	0.064 bc	13.0 ab
DOR 364 (nod)	2377 abc	603.7 bcd	18.87 b	1.64 c	0.227 c	0.008 c	6.06 bc
Mean	2725	823.8	42.38	4.55	0.545	0.058	8.889

\*\* , \* , +. Indicates significance at 0.01, 0.05, and 0.10, respectively, according to DMRT.

Table 9. Total RL (cm) of root width classes 1, 2, 3, 4, 5, and 10 at 15.3 - 30.5 cm depth for nine common bean (*Phaseolus vulgaris* L.) genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI, at 27°C ± 2 day/night temperatures and a 15 h photoperiod under moisture stress conditions. n = 3.

Genotypes	Class 1 0 - 0.5 mm	Class 2 0.51 - 1.0 mm	Class 3 1.01 - 1.5 mm	Class 4 1.51 - 2.0 mm	Class 5 2.01 - 2.5 mm	Class 10 > 4.5 mm
BAT 477 (nod)	8273 bc*	1872 bc**	675.99 bc+	10.92 d*	1.47 c+	25.01 bc**
PR9603-22	8752 abc	2435 bc	810.62 abc	11.94 cd	1.49 c	26.46 abc
DOR 364 (nn)	5701 c	1346 c	470.56 c	8.669 d	1.93 bc	18.60 c
ICA Palmar	10590 ab	2632 abc	821.49 abc	19.81 abc	3.92 a	35.67 ab
XAN 176	12358 ab	2984 ab	1006.6 ab	20.56 ab	3.42 ab	36.60 ab
BAT 477 (nn)	12199 ab	2538 bc	947.65 ab	15.81 abcd	2.49 abc	35.70 ab
SEA5	12897 a	3924 a	1160.8 a	22.90 a	3.71 a	39.62 a
8-42-M-2	11964 ab	2433 bc	810.23 abc	16.36 abcd	2.83 abc	36.80 ab
DOR 364 (nod)	9086 abc	2063 bc	643.27 bc	14.12 bcd	3.14 abc	24.61 bc
Mean	10202	2470	816.36	15.68	2.71	31.01

\*\*\*, \*, +. Indicates significance at 0.01, 0.05, and 0.10, respectively, according to DMRT.



Table 10. Root length (cm) of nine common bean (*Phaseolus vulgaris* L.) genotypes of root width classes 1, 2, 3, 4, and 10 at 15.3 - 30.5 cm depth grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under moisture stress conditions. Means ± SE, n = 3.

Genotypes	Class 1 0 - 0.5 mm	Class 2 0.51 - 1.0 mm	Class 3 1.01 - 1.5 mm	Class 4 1.51 - 2.0 mm	Class 10 > 4.5 mm
Stress treatment					
BAT 477 (nod)	2028 bc**	324.30 c**	28.22 abc**	2.41 bc+	5.91 bc*
PR9603-22	2255 bc	335.72 bc	18.44 c	1.75 c	5.53 c
DOR 364 (nn)	1868 c	312.65 c	18.57 c	1.48 c	5.65 c
ICA Palmar	2192 bc	405.02 bc	32.02 abc	5.10 a	7.03 abc
XAN 176	3605 a	617.55 a	38.65 a	4.69 ab	9.34 ab
BAT 477 (nn)	3186 ab	411.55 bc	38.12 a	3.33 abc	9.24 ab
SEAS	2036 bc	489.27 abc	27.10 abc	3.13 abc	6.70 abc
8-42-M-2	3189 ab	520.43 ab	35.78 ab	4.70 ab	9.80 a
DOR 364 (nod)	2006 bc	312.60 c	23.97 bc	4.60 ab	4.97 c
Mean	2485	414.34	28.98	3.47	7.13
Nonstress treatment					
BAT 477 (nod)	4831 ns‡	869.05 ns	48.01 ns	6.16 ns	14.14 ns
PR9603-22	4029	1113.0	56.63	8.56	14.20
DOR 364 (nn)	3351	663.55	40.33	5.03	11.20
ICA Palmar	4618	838.68	78.12	8.79	12.64
XAN 176	4147	948.17	80.00	13.4	12.44
BAT 477 (nn)	4946	925.23	48.92	4.25	13.84
SEAS	2417	588.39	44.66	5.13	8.056
8-42-M-2	3375	766.86	64.40	8.38	11.64
DOR 364 (nod)	4203	937.88	44.63	4.28	10.90
Mean	3991	850.06	56.19	7.11	12.12

\*\* , \* , +. Indicates significance at 0.01, 0.05, and 0.10, respectively, according to DMRT.

‡ Indicates no significant difference among means within a column.

Table 11. Root length (cm) for root width classes 1, 2, 3, 4, 5, 6, and 10 at a depth of 45.8 - 61 cm for nine common bean (*Phaseolus vulgaris* L.) genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI, at 27°C ± 2 day/night temperatures and a 15 h photoperiod under moisture stress conditions. n = 3.

Genotypes	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 10
	0 - 0.5 mm	0.51 - 1.0 mm	1.01 - 1.5 mm	1.51 - 2.0 mm	2.01 - 2.5 mm	2.51 - 3.0 mm	> 4.5 mm
BAT 477 (nod)	1055 cd+	467.5 c*	20.65 b**	1.83 bc**	0.214 b*	0.006 b**	5.03 cd+
PR9603-22	1317 bcd	639.3 bc	22.76 b	1.86 bc	0.101 b	0.022 b	6.84 bcd
DOR 364 (nn)	212.0 d	223.6 c	5.893 b	0.65 c	0.011 b	0.000 b	2.08 d
ICA Palmar	1826 bcd	655.6 bc	33.53 ab	3.94 bc	0.698 ab	0.046 b	8.86 abc
XAN 176	3555 ab	1123 ab	45.58 ab	5.68 ab	0.609 b	0.044 b	11.5 ab
☼ BAT 477 (nn)	2180 abcd	522.7 bc	23.70 b	1.97 bc	0.172 b	0.004 b	6.08 bcd
SEA5	4159 a	1344 a	65.63 a	8.86 a	1.318 a	0.206 a	13.9 a
8-42-M-2	2460 abcd	521.8 bc	21.38 b	1.92 bc	0.131 b	0.030 b	6.90 bcd
DOR 364 (nod)	2858 bcd	802.6 abc	24.69 b	2.39 bc	0.396 b	0.016 b	7.43 bcd
Mean	2180	700	29.31	3.23	0.406	0.042	7.63

\*\* , \* , +. Indicates significance at 0.01, 0.05, and 0.10, respectively, according to DMRT.



Table 12. Total root dry weight (RDW), root length (RL), average root diameter (RD), average root surface area (RSA), average root volume (RV), and root length density (RLD), for all root width classes of common bean plants grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed conditions. n = 27.

	RDW (g)	RL (m)	RSA (cm <sup>2</sup> )	RD (mm)	RV (cm <sup>3</sup> )	RLD (cm cm <sup>-3</sup> )
<u>0 - 15.2 cm depth</u>						
Stress	0.10***	8.73**	96.7**	0.33 ns‡	0.88***	0.07**
Nonstress	0.41	33.33	371.9	0.32	3.55	0.27
<u>15.3 - 30.5 cm depth</u>						
Stress	0.20*	29.40*	289.6*	0.29**	2.48*	0.24*
Nonstress	0.35	49.19	517.2	0.31	4.72	0.40
<u>30.6 - 45.7 cm depth</u>						
Stress	0.25 ns	40.95 ns	432.7 ns	0.30 ns	4.0 ns	0.34 ns
Nonstress	0.36	59.92	642.8	0.30	6.0	0.49
<u>45.8 - 61 cm depth</u>						
Stress	0.17 ns	33.17 ns	372.7 ns	0.27 ns	3.6 ns	0.27 ns
Nonstress	0.27	42.90	523.6	0.25	4.8	0.35
<u>61.1 - 76.2 cm depth</u>						
Stress	0.10 ns	18.56 ns	217 ns	0.14 ns	2.2 ns	0.15 ns
Nonstress	0.15	23.32	291	0.14	3.1	0.19
<u>76.3 - 92 cm depth</u>						
Stress	0.01+	1.30 ns	19.2 ns	0.07*	0.24 ns	0.01 ns
Nonstress	0.02	1.65	26.0	0.11	0.34	0.01

\*\*\*, \*\*, \*, + Indicates significant difference among means within a column at P ≤ 0.001, 0.01, 0.05, and 0.10, respectively, according to DMRT.

‡ Indicates no significant difference among means within a column.

Table 13. Root length density (RLD) ( $\text{cm cm}^{-3}$ ) for nine genotypes of common bean (*Phaseolus vulgaris* L.) plants grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at  $27^{\circ}\text{C} \pm 2$  day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions at two soil depths, 15.3 to 30.5 and 45.8 to 61 cm. n = 3 (stress and nonstressed RLD) and 6 (combined RLD).

	RLD ( $\text{cm cm}^{-3}$ )				
	Stressed	Non-stressed	Combined	Stressed	Non-stressed
	-----15.3 - 30.5 cm-----		-----45.8 - 61 cm-----		
BAT 477 (nod)	0.20 bc**	0.47 ns§	0.17 c+	0.20 ns	0.14 ns
PR9603-22	0.22 bc	0.43	0.27 abc	0.25	0.28
DOR 364 (nn)	0.18 c	0.33	0.18 c	0.10	0.26
ICA Palmar	0.22 bc	0.46	0.30 abc	0.29	0.30
XAN 176	0.35 a	0.43	0.48 a	0.39	0.57
BAT 477 (nn)	0.30 abc	0.49	0.30 abc	0.22	0.38
SEA5	0.21 bc	0.25	0.41 ab	0.46	0.35
8-42-M-2	0.31 ab	0.35	0.47 ab	0.25	0.69
DOR 364 (nod)	0.19 bc	0.43	0.25 bc	0.30	0.20
Mean	0.24	0.40	0.31	0.27	0.35

\*\* , +. Indicates significant difference among means within a column at  $P \leq 0.01$  and 0.10, respectively, according to DMRT.

§ Indicates no significant difference among means within a column.

Table 14. Dry weight (g) of shoot and root and root/shoot ratio of eight common bean genotypes germinated in a germination chamber for 4 d at 25°C and transplanted to an environmentally controlled growth chamber for 28 d at 23/20°C day/night temperatures and a 15 h photoperiod and grown under control conditions in half-strength Hoagland's solution or in 10<sup>-6</sup> M ABA solution. n = 32.

<b>Treatment</b>	<b>14 DAT</b>	<b>21 DAT</b>	<b>28 DAT</b>
		<b><u>Shoot</u></b>	
ABA	0.373 ns‡	0.659*	0.976 ns
Control	0.373	0.522	0.858
		<b><u>Root</u></b>	
ABA	0.139 ns	0.264**	0.395 ns
Control	0.124	0.186	0.296
		<b><u>R/S ratio</u></b>	
ABA	0.373 ns	0.398*	0.418 <sup>+</sup>
Control	0.337	0.357	0.347

‡ Indicates no significant difference among means within a column.

\*\* , \* , + Indicates significant difference among means within a column at  $P \leq 0.01, 0.05,$  and 0.10, respectively, according to DMRT.

Table 15. Shoot and root dry weight (g) and root/shoot ratio of eight common bean genotypes germinated in a germination chamber for 4 d at 25°C and transplanted to an environmentally controlled growth chamber for 28 d at 23/20°C day/night temperatures and a 15 h photoperiod, grown in a half-strength Hoagland's nutrient solution, and sampled at 14, 21, and 28 DAT. Control treatment. N = 4.

Genotypes	14 DAT			21 DAT			28 DAT		
	Shoot	Root	R/S ratio	Shoot	Root	R/S ratio	Shoot	Root	R/S ratio
BAT 477 (nod)	0.32 b**	0.12 ab*	0.37 ab**	0.42 c+	0.14 b**	0.35 bc**	0.85 abc*	0.23 ns†	0.32 ns
PR9603-22	0.42 ab	0.11 ab	0.27 b	0.68 ab	0.18 ab	0.25 c	0.93 abc	0.23	0.25
DOR 364 (nn)	0.28 b	0.08 b	0.30 ab	0.52 bc	0.15 b	0.30 bc	0.75 bc	0.24	0.32
XAN 176	0.56 a	0.16 a	0.30 ab	0.77 a	0.30 a	0.39 b	1.22 a	0.43	0.35
BAT 477 (nn)	0.31 b	0.11 ab	0.36 ab	0.42 c	0.16 b	0.38 b	0.87 abc	0.32	0.35
SEA5	0.41 ab	0.15 a	0.38 a	0.54 bc	0.29 a	0.53 a	0.63 c	0.27	0.40
8-42-M-2	0.39 ab	0.15 a	0.37 a	0.45 bc	0.14 b	0.32 bc	0.51 c	0.23	0.40
DOR 364 (nod)	0.30 b	0.11 ab	0.37 a	0.37 c	0.12 b	0.33 bc	1.12 ab	0.40	0.38
Mean	0.37	0.12	0.34	0.52	0.19	0.36	0.86	0.30	0.35

\*\* , \* , +. Indicates significant difference among means within a column at  $P \leq 0.01$ , 0.05, and 0.10, respectively, according to DMRT

† Indicates no significant difference among means within a column.

Table 16. Shoot and root dry weight (g) and root/shoot ratio of eight common bean genotypes germinated in a germination chamber for 4 d at 25°C and transplanted to an environmentally controlled growth chamber for 28 d at 23/20°C day/night temperatures and a 15 h photoperiod, grown in a 10<sup>-6</sup> M ABA, and sampled at 14, 21, and 28 DAT. n = 4. ABA treatment.

Genotypes	-----14 DAT-----			-----21 DAT-----			-----28 DAT-----		
	Shoot	Root	R/S ratio	Shoot	Root	R/S ratio	Shoot	Root	R/S ratio
BAT 477 (nod)	0.30 ns‡	0.11 ns	0.36 abc**	0.56 ns	0.21 b*	0.39 abc*	0.69 bc**	0.27 c**	0.40 abc+
PR9603-22	0.39	0.11	0.27 c	0.85	0.31 ab	0.37 bc	0.80 bc	0.28 c	0.34 bc
DOR 364 (nn)	0.36	0.12	0.32 bc	0.72	0.24 b	0.34 c	1.44 a	0.42 abc	0.30 c
XAN 176	0.42	0.12	0.28 c	0.59	0.25 ab	0.39 abc	1.23 ab	0.49 ab	0.41 abc
BAT 477 (nn)	0.40	0.16	0.40 ab	0.58	0.18 b	0.31 c	0.85 bc	0.35 bc	0.42 abc
SEA5	0.41	0.18	0.43 ab	0.84	0.40 a	0.48 a	1.01 abc	0.50 ab	0.52 a
8-42-M-2	0.42	0.19	0.46 a	0.72	0.33 ab	0.46 ab	1.15 abc	0.58 a	0.51 a
DOR 364 (nod)	0.30	0.13	0.46 a	0.42	0.19 b	0.45 ab	0.64 c	0.26 c	0.45 ab
Mean	0.37	0.14	0.37	0.66	0.26	0.40	1.0	0.40	0.42

‡ Indicates no significant difference among means within a column.

\*\* , \* , +. Indicates significant difference among means within a column at P ≤ 0.01, 0.05, and 0.10, respectively, according to DMRT

Table 17. Dry weight (g) of leaves, stems, reproductive parts, shoots, and root and root/shoot ratio of nine common bean genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at  $27^{\circ}\text{C} \pm 2$  day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions.  $n = 27$ .

	Leaf	Stem	Repro.	Shoot	Root	R/S ratio
Stress	3.41***	2.72**	0.68*	5.85***	0.84*	0.17+
Non-stress	9.75	4.33	1.32	16.4	1.60	0.10

\*\*\*, \*\*, \*, +. Indicates significant difference among means within a column at  $P \leq 0.001, 0.01, 0.05,$  and  $0.10$  respectively, according to DMRT

Table 18. Dry weight (g) of leaves, stems, reproductive parts, shoots, and root and root/shoot ratio of nine common bean genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions. n = 6.

Genotypes	Leaf	Stem	Repro.	Shoot	Root	R/S ratio
BAT 477 (nod)	7.04 ns‡	3.63 ns	1.73 ab***	12.4 ns	1.22 abc+	0.12 ns
PR9603-22	5.66	4.53	1.55 abc	11.7	1.14 abc	0.10
DOR 364 (nn)	5.76	3.00	0.59 bcd	9.31	0.83 c	0.17
ICA Palmar	7.26	2.91	0.00 d	10.2	1.20 abc	0.14
XAN 176	6.71	4.06	0.32 cd	11.1	1.51 a	0.14
BAT 477 (nn)	7.90	3.43	1.05 bcd	12.4	1.24 ab	0.12
SEA5	5.93	2.83	2.61 a	11.4	1.45 a	0.15
8-42-M-2	7.10	4.30	0.58 bcd	12.0	1.30 ab	0.14
DOR 364 (nod)	5.86	3.20	0.56 bcd	9.62	1.00 bc	0.11
Mean	6.58	3.54	1.00	11.13	1.20	0.13

‡ Indicates no significant difference among means within a column.

\*\*\*, + Indicates significant difference among means within a column at  $P \leq 0.001$  and 0.10, respectively, according to DMRT.

Table 19. Dry weight (g) of leaves, stems, reproductive parts, shoots, and root and root/shoot ratio of nine common bean genotypes grown in 0.92 m polyvinyl chloride (PVC) tubes of 30 cm diameter in a glasshouse at Michigan State University, East Lansing, MI. at 27°C ± 2 day/night temperatures and a 15 h photoperiod under stressed and non-stressed moisture conditions. n = 3.

Genotypes	Stress treatment							Nonstress treatment						
	Leaf (g)	Stem (g)	Repro. (g)	Shoot (g)	Root (g)	R/S	Leaf (g)	Stem (g)	Repro. (g)	Shoot (g)	Root (g)	R/S		
BAT 477 (mod)	2.95ns†	1.23 cd+	0.77 b**	4.95 ns	0.70 cd**	0.16 ns	11.1 ns	6.04 ns	2.70 ab*	19.9 ns	1.7 ns	0.09 de+		
PR9603-22	3.38	2.57 a	1.16 ab	7.10	0.74 bcd	0.11	7.94	6.48	1.94 abc	16.4	1.6	0.10 cd		
DOR 364 (nn)	2.06	0.98 d	0.26 b	3.31	0.50 d	0.27	9.46	4.95	0.91 bc	15.3	1.2	0.08 e		
ICA Palmar	4.16	1.49 bcd	0.00 b	5.64	0.88 bc	0.16	10.4	4.34	0.00 c	14.7	1.5	0.11 bc		
XAN 176	3.47	2.15 ab	0.22 b	5.84	0.86 bc	0.15	9.94	6.00	0.43 c	16.3	2.2	0.13 a		
BAT 477 (nn)	4.52	2.11 ab	0.65 b	7.28	1.01 ab	0.15	11.3	4.76	1.44 abc	17.5	1.5	0.09 de		
SEAS	3.31	1.58 bcd	2.27 a	7.17	1.22 a	0.19	8.55	4.07	2.94 a	15.6	1.7	0.12 b		
8-42-M-2	3.22	1.90 abc	0.31 b	5.43	0.92 bc	0.18	11.0	6.67	0.84 bc	18.5	1.7	0.09 de		
DOR 364 (mod)	3.62	1.91 abc	0.43 b	6.00	0.74 bcd	0.13	8.10	4.49	0.70 c	13.3	1.2	0.09 de		
Mean	3.41	1.77	0.68	5.85	0.84	0.17	9.75	5.31	1.32	16.4	1.65	0.10		

† Indicates no significant difference among means within a column.

\*\* , \* , + Indicates significant difference among means within a column at P ≤ 0.01, 0.05, and 0.10, respectively, according to DMRT.



### Chapter 3

#### **Nitrogen fixation and partitioning of nine Caribbean and Central American common bean (*Phaseolus vulgaris* L.) lines grown under rainfed and glasshouse conditions.**

##### **Abstract**

Common bean is grown on more than 12 million ha and constitutes the most important food legume for more than 500 million people in Latin America, the Caribbean, and Africa. This study was conducted to assess genotypes for N fixation, N-use efficiency, and N harvest index. Field studies were conducted in 1999 and 2000 at the Agricultural Experiment Station in St. Croix, United States Virgin Islands to determine the N<sub>2</sub> fixing capacity of nine common bean lines grown under irrigated and rainfed field conditions. The total N difference method was used to estimate N<sub>2</sub> fixation with non-nodulating (nn) isolines of BAT 477 and DOR 364 as the reference crops. BAT 477 [nodulating (nod)] was one of the genotypes with the highest root-N concentration. ICA Palmar had the highest stem-N concentration. Leaf-N concentration was highest in 8-42-M-2 in 1999 and in DOR 364 (nn) in 2000. Nitrogen HI values among genotypes ranged from 7 to 76%. Nitrogen use efficiency did not differ among irrigated and rainfed treatments in 1999 but was greater in the irrigated treatment in 2000. XAN 176 produced a high NUE and high NHI. Total N-fixed among the genotypes was low and ranged from no fixation (-34.3 kg ha<sup>-1</sup>) to 19.9 kg ha<sup>-1</sup>, with DOR 364 (nod) producing the highest numerical fixation both years. The reference crop DOR 364 (nn) gave a higher estimate of N-fixation than did BAT 477 (nn).

## Introduction

Many important common bean (*Phaseolus vulgaris* L.) producing areas of the world experience moisture deficits during the growing season (Ehleringer et al., 1991), thus emphasizing the need to breed for drought resistance. Such efforts can be greatly aided by an increased understanding of the physiological responses which enable beans to survive drought with minimal yield reductions. Beans have a high protein content, approximately 26% (Bressani and Elias, 1980), and most of the N in the crop at harvest is located in the seed. Therefore, it is important to know the impact that water deficits may have on N partitioning and remobilization.

A number of studies have evaluated the importance of symbiotic N fixation in the N economy of bean, based on total N acquisition and the allocation of symbiotically fixed N to reproductive and vegetative tissues (Dubois and Burris, 1986; Kucey, 1989; Rennie and Kemp, 1984; Lynch and White, 1992; Foster et al., 1995). Westermann et al. (1985) reported general N distribution patterns in bean plants grown in small pots of perlite in a greenhouse.

In the tropics, soil organic matter declines rapidly with cultivation (Boddey et al., 1997; Vlek et al., 1997). Where inputs are limited, changes in soil organic matter following cultivation quickly lead to low soil fertility and to diminished soil structure, water holding capacity, and biological activity (Vlek et al., 1997). Studies conducted in temperate environments may not apply to tropical environments, which differ significantly from temperate environments with regard to photoperiod, temperature, humidity, and radiation, all of which might influence senescence and N allocation (Lynch

and White, 1992).

Because soil N deficiency is common in the tropics and subtropics (Graham, 1981; Dakora and Keya, 1997), N supply, N management, and N-use efficiency are significant factors in crop production in these regions and spark intermittent concern about the availability of fossil fuel reserves for future fertilizer N production (Graham and Vance, 2000). The objective of this study was to investigate N fixation among several Caribbean and Central American common bean genotypes under limiting and non-limiting moisture regimes. This information may be useful in identifying opportunities for genetic improvement of N-use efficiency of common bean lines for Latin America, the Caribbean, and Africa.

## **Materials and methods**

### ***Field Study***

Two experiments were conducted at the Agricultural Experiment Station, University of the Virgin Islands, Kingshill, St. Croix, United States Virgin Islands (U.S.V.I.) in 1999 and 2000. Mean air temperature was 26.1<sup>o</sup> C. Seeds were planted on 9 March and harvested on 1 June 1999 and on 6 April and harvested on 27 June 2000 (stress plots) and on 30 June 2000 (non-stress plots). The soil at the Agricultural Experimental Station field site is classified as a Fredensborg loamy, fine carbonatic, isohyperthermic, shallow, Typic Calciustoll with pH ranging from 7.6 to 8.4.

In 1999, soil samples from each plot were taken and analyzed by the Michigan

State University Plant Nutrient and Soil Testing Laboratory for N, P, K, Zn, Mn, and Cu. As indicated by the soil analysis, 22 kg P/ha<sup>-1</sup>, 5.6 kg Zn/ha<sup>-1</sup>, and 10 kg Mn/ha<sup>-1</sup> were applied in 1999 and 2000. No N fertilizer was applied, since N fixation was being assessed. Samples from each block (stress and non-stress) were taken in 2000.

In 1999, applications of insecticide, Sevin 80WP (0.68 kg ai/A) and Diazinon AG500 (170 g ai/A), were made at one week intervals starting on 26 March to control bean leafskeletonizer. One application of fungicide, Benomyl (500g per 95 L/A) and M-Pede (Potassium salts of fatty acids) (71 g per 3.8 L/A) was made on 18 April for control of *Cercospora* (*Cercospora canescens*). No insecticides or fungicides were applied to field plots in 2000.

### ***Plant material***

Nine common bean genotypes possessing Type I, II, or III growth habits (Table 1) were included in this study: BAT 477 [nodulating (nod) and non-nodulating (nn)], DOR 364 [nodulating (nod) and non-nodulating (nn)], XAN 176, ICA Palmar, 8-42-M-2, SEA5, and PR9603-22, local check (obtained from Dr. James Beaver, University of Puerto Rico-Mayaguez Campus). BAT 477 (nod) and 8-42-M-2 were the drought resistant and drought susceptible checks, respectively. Seeds were inoculated with a granular form of *Rhizobium etli*, which was applied within the furrow.

### ***Experimental design***

The study utilized a randomized complete block design with four replications, moisture as the main plot, and genotype as subplot. In 1999, seeds were planted into four-row plots of 0.5 m row spacing and 2.48 m length. Each row was planted at a

density of 25 seeds and thinned to 23 plants. In 2000, seeds were planted into four-row plots of 0.5 m row spacing and 2.13 m length and planted at a density of two seeds per station at 7.62 cm between stations. Moisture stress was initiated at the V3 growth stage or 20 DAP (days after planting) by cessation of irrigation to the rainfed plots. Control plots were maintained at a soil moisture content of -30 kPa.

### ***Data collection***

In 1999, plants were sampled at V3, R2, and R7 and in 2000 at V3, R4, and R8 growth stages (Nuland and Schwartz, 1989). At sampling, three plants per plot were extracted from the soil, dipped in water to remove all soil and debris, and separated into leaves, stems, roots, and reproductive parts (flowers and/or pods) for dry weight and total N determination. Nitrogen was determined by Kjeldahl digestion and total N analysis was done using the Lachat procedure. Dry plant material was ground in a Udy cyclone sample mill (Udy Corporation, Fort Collins, Co.) to pass through a 2-mm screen. Plant samples of 0.1 g were digested in 4 ml of 18 M H<sub>2</sub>SO<sub>4</sub> with 1.5 g K<sub>2</sub>SO<sub>4</sub> and 0.075 g Se catalyst.

### ***N<sub>2</sub> fixation***

Nitrogen fixation was determined by the difference method. Two non-nodulating bean lines [BAT 477 (nn) was obtained from Dr. Steven Beebe (CIAT) and DOR 364 (nn) from Dr. James Beaver (University of Puerto Rico-Mayaguez campus)] were utilized as reference crops. The difference in the total N accumulated by the nodulating lines and the non-nodulating control is regarded as the amount of N<sub>2</sub> fixed (Smith and Hume, 1987;

Ayisi et al., 1992; Ohdan and Daimon, 1998). Thus:  $N_2 \text{ fixed} = N_{\text{fixed by nodulating line}} - N_{\text{fixed by non-nodulating line}}$ . The major assumption of this method is that the nodulating lines and the non-nodulating lines take up identical amounts of N from the soil.

### ***Glasshouse study***

Plants were grown in polyvinyl chloride tubes (PVC) for 40 days in a glasshouse at Michigan State University, in East Lansing, MI. The temperature regime was  $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and light intensity was  $1421 \mu\text{E m}^{-2}\text{s}^{-1}$  with a 15 h photoperiod. The experimental design was a split plot with water (stressed and non-stressed) as the main plot, genotypes as the subplot, and three replications. The PVC tubes were one meter in length with a diameter of 30.5 cm. Five seeds per PVC tube were planted on 7 August, 2000 and thinned to one plant per PVC tube at 14 days after planting (DAP). Stress was initiated at 14 DAP by cessation of water to plants in the stress treatment. Plants were sampled at R1/2 growth stage (40 DAP). Stem, leaf, and reproductive parts were weighed and dried at  $60^{\circ}\text{C}$  for 48 h, re-weighed and then ground to pass through a 2 mm screen Udy Cyclone Sample Mill (Udy Corporation, Fort Collins, CO.) And analyzed for total nitrogen.

### ***Statistics***

The MSTAT micro-computer statistical package (Michigan State University) for agricultural sciences was used for all data analysis.

## **Results and discussion**

### ***N-Partitioning***

### ***Water effect***

Moisture stress did not significantly affect root-N concentration at any growth stage in either year (Table 1). There were no significant differences in stem-N concentration between the irrigated and rainfed moisture conditions of the field study on any of the sampling dates except at the V3 growth stage ( $P \leq 0.10$ ) in 2000 where irrigated plants had a lower stem-N concentration (Table 1). Leaf-N was significantly lower under rainfed conditions than under the irrigated treatment at the R7 growth stage in 1999 and significantly higher on all sampling dates in 2000. There were no significant differences in leaf-N concentration between stressed and nonstressed moisture treatments in the PVC experiment (Table 1). Reproductive-N was greater in the irrigated treatment than rainfed at the R7 ( $P \leq 0.05$ ) growth stage in 1999 and in the rainfed treatment at the R4 ( $P \leq 0.05$ ) growth stage in 2000 (Table 1).

#### ***Root-N concentration***

In 1999, there were significant genotypic differences in root-N concentration in the combined stress and nonstress treatments at the V3, R2, and R7, at R2 and R7 under rainfed conditions, and at V3 and R7 under irrigated conditions (Table 2). In the combined analysis at the V3 growth stage, the genotype BAT 477 (nod) had a significantly higher ( $P \leq 0.05$ ) root-N concentration than the genotypes DOR 364 (nn), ICA Palmar, and BAT 477 (nn). At the R2 growth stage, BAT 477 (nod) had a significantly higher root N-concentration ( $P \leq 0.05$ ) than the genotypes PR9603-22, DOR 364 (nn), and XAN 176. At the R7 growth stage, the genotype ICA Palmar, had a significantly higher ( $P \leq 0.001$ ) root-N concentration than all other genotypes except BAT 477 (nod) (Table 2).

In the rainfed treatment at the R2 growth stage, BAT 477 (nod) had a significantly higher root-N concentration than PR9603-22, DOR 364 (nn), and XAN 176. At the R7 growth stage of the rainfed treatment, BAT 477 (nod) was significantly higher than all other genotypes except ICA Palmar (Table 2). In the irrigated treatment, BAT 477 (nod) root-N concentration was higher than PR9603-22, DOR 364 (nn), ICA Palmar and BAT 477 (nn) at both V3 ( $P \leq 0.01$ ) and R7 ( $P \leq 0.05$ ) (Table 2).

In 2000, genotypic root-N concentration was significantly at the R4 growth stage (Table 3). The genotype ICA Palmar had a significantly higher ( $P \leq 0.10$ ) root-N concentration than the genotypes XAN 176 and SEA5 in the combined analysis at the R4 growth stage (Table 3). In the rainfed treatment at R4 growth stage, SEA5 had a significantly lower root-N concentration than all the genotypes except XAN 176 and BAT 477 (nn) (Table 3). Overall, BAT 477 (nod) had a high root-N concentration and there was a tendency for a higher root-N concentration in BAT 477 (nod) than in BAT 477 (nn) and in DOR 364 (nn) than in DOR 364 (nod). Interestingly, SEA5 and XAN 176 had lower root-N concentration, but a high RLD (Chapter 2). While XAN 176, PR9603-22, and DOR 364 (nn) generally had a low root N-concentration, but relatively high yield.

#### ***Stem-N concentration***

In 1999, genotypic stem-N concentration was significantly different only at the R7 growth stage in the combined, rainfed, and irrigated analysis, with the genotype ICA Palmar having a significantly higher ( $P \leq 0.001$ ) concentration than all the other genotypes and XAN 176 one of the lowest (Table 4). In 2000, there were no significant



differences between the genotypes for the combined and rainfed treatments of the field study but there were significant differences at the R8 growth stage in the irrigated treatment (Table 5) with the genotype ICA Palmar having a significantly higher ( $P \leq 0.10$ ) stem-N concentration than all other genotypes (Table 5). In the PVC experiment, the genotype ICA Palmar again had the highest stem-N concentration but was only statistically different than the genotype PR9603-22 in the combined analysis and than PR9603-22 and 8-42-M-2 in the nonstress treatment (Table 5).

### ***Leaf-N concentration***

In 1999 at the R2 growth stage in the combined analysis, the genotypes 8-42-M-2 and ICA Palmar had a significantly higher ( $P \leq 0.05$ ) leaf-N concentration than the genotypes BAT 477, DOR 364 (nn), ICA Palmar, and SEA5. DOR 364 (nod) had a higher leaf-N concentration than its non-nodulating isolate. At the R7 growth stage, 8-42-M-2 and ICA Palmar had a significantly higher leaf-N concentration ( $P \leq 0.001$ ) than PR9603-22, DOR 364 (nn), and DOR 364 (nod) (Table 6). In the rainfed treatment, DOR 364 (nod) had one of the highest ( $P \leq 0.10$ ) leaf-N concentrations at the R2 growth stage and the genotype ICA Palmar had one of the highest ( $P \leq 0.01$ ) leaf N-concentration at the R7 growth stage (Table 6). In the irrigated treatment, the genotype SEA5 was among the genotypes with the highest leaf-N at the V3 growth stage and the genotype 8-42-M-2 was among the highest at both R2 and R7 growth stages (Table 6). In 2000 significant genotypic differences were only observed at the V3 growth stage for combined, rainfed, and irrigated analyses of the field study at the UVI field trial (Table 7). The genotype DOR 364 (nn) had one of the highest leaf-N concentrations in all three analyses (Table

7). There were no significant genotypic differences observed in the PVC experiment for the combined and nonstress analyses but in the stress analyses the genotype DOR 364 (nn) again had one of the highest leaf-N concentrations but was not statistically different from the genotype XAN 176 (Table 7).

#### ***Reproductive-N concentration***

In the combined analyses at the R2 growth stage in 1999, the genotype DOR 364 (nod) had significantly higher ( $P \leq 0.001$ ) combined pod and seed-N concentrations than all of the other genotypes, but had one of the lowest pod-N and seed N-concentrations by the R7 growth stage (Table 8). At the R7 growth stage in 1999, ICA Palmar had a significantly higher ( $P \leq 0.001$ ) pod N concentration than the genotypes PR9603-22, DOR 364 (nn), and XAN 176. In the rainfed treatment, the genotype ICA Palmar had one of the highest pod-N concentrations at both R2 and R7 growth stages and SEA5 had one of the highest seed-N concentration at R7 (Table 8a). In the irrigated treatment, the genotype DOR 364 (nod) had one of the highest pod-N concentrations at the R2 growth stage and the genotype BAT 477 (nod) had one of the highest at the R7 growth stage (Table 8b). In 1999, there was a tendency for DOR 364 (nod) to have a higher reproductive-N concentration than DOR 364 (nn) but for BAT 477 (nod) to have a lower reproductive-N concentration than BAT 477 (nn). Similarly, BAT 477 (nn) tended to be one of the genotypes with a higher reproductive-N concentration, while DOR 364 (nn) tended to be one of the lines with a lower reproductive-N concentration. The relative N fixation using BAT 477 (nn) and DOR 364 (nn) reflect these trends.

In 2000, there were no significant genotypic differences in reproductive-N

concentration in the combined and irrigated analyses of the field study but seed-N was significantly different in the rainfed analyses at the R8 growth stage with the genotype XAN 176 having one of the lowest concentrations (Table 9). In the PVC experiment, the genotype XAN 176 had a significantly higher ( $P \leq 0.001$ ) reproductive-N concentration at the R2 growth stage than all other genotypes in both combined and stressed analyses, except the genotypes BAT 477 (nn), 8-42-M-2, and DOR 364 (nod) (Table 8). However, in the nonstress analyses, XAN 176 was only significantly higher ( $P \leq 0.01$ ) than PR9603-22 (Table 9).

Nitrogen flow from pods could also have been a significant source of seed N. The decline in pod-N concentration differs with the observations of Oliker et al (1978), who concluded that N in pod walls was not available for seed growth of common bean and in fact proposed that pod walls competed with seeds for N. This discrepancy may be due to the fact that Oliker et al. (1978) used a snap bean genotype in their studies. Snap beans have been selected for pod development rather than seed development and may have very different N allocation patterns than common bean genotypes.

#### ***Nitrogen harvest index (NHI)***

Nitrogen harvest index (NHI) was significantly reduced ( $P \leq 0.01$ ) in 1999 in the nonstress moisture treatment, but in 2000 there was no significant difference between moisture treatments. This contrasted with results reported by Foster et al. (1995), who observed a reduced NHI under moisture stressed conditions, the 1999 results may reflect the common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*) and *Cercospora* (*Cercospora canescens*) infestations that heavily affected the non-stress plots (Chapter 1).

There were significant combined, rainfed, and irrigated genotypic differences for NHI in 1999 but not in 2000 (Table 10). In 1999, the genotype DOR 364 had a significantly higher ( $P \leq 0.001$ ) combined NHI than the genotypes 8-42-M-2, SEA5, and ICA Palmar and the genotypes XAN 176 and PR9603-22 had a significantly higher ( $P \leq 0.001$ ) NHI in the rainfed treatment than ICA Palmar, BAT 477 (nn), and SEA5 but not significantly different than the other genotypes (Table 10). In the irrigated treatment, the genotype DOR 364 (nod) again had the highest NHI but was only statistically different ( $P \leq 0.01$ ) from ICA Palmar and 8-42-M-2 (Table 10).

Nitrogen harvest index values among the genotypes ranged from 7 to 76%. These results are in the range reported previously for NHI reported by Foster et al. (1995) (*P. vulgaris*); Thomas and Hungria (1988) (*P. vulgaris*); and Brunner and Zapata (1984) (*V. faba*), who all reported ranges from a low of 18% to a high of 91 percent. There was a highly positive significant correlation between seed weight and NHI in 1999 (0.90\*\*\*) and 2000 (0.81\*\*\*). Also, significant correlations occurred between NHI and harvest index (HI) in 1999 (0.99\*\*\*) and in 2000 (0.97\*\*\*). High NHI represents the increased capacity of a genotype to mobilize and translocate nitrogen from the leaves, stems, and roots to the seed of the plant.

Nitrogen harvest index, which is a measure of the total nitrogen content of the seeds versus that in the portion of the plant above the ground, has been shown to be subject to considerable variation. The capability of effective transfer of nitrogen from above ground biomass to the seed has been documented in many crops [wheat (*Triticum aestivum*) McKendry et al. (1988); Desai and Bhatia (1978), oats (*Avena sativa*) Kairudin

and Frey (1990); Rattunde and Frey (1986), corn (*Zea mays*) Katsantonis et al. (1988), and soybean (*Glycine max*) Shiraiwa and Hashikawa (1995); Chandel et al. (1989)]. These studies have presented values for NHI as high as 80-84%. In these crops, it is doubtful whether a further portion of the nitrogen remaining in the aboveground biomass could be transferred to the seed.

### ***Harvest index (HI)***

The harvest index (HI) represents the increased capacity of a plant to translocate photosynthates from other plant organs to the seed (Donald and Hamblin, 1976). In 1999, harvest index was significantly increased in the moisture stress treatment ( $P \leq 0.05$ ) but not in 2000, although there was a tendency for this to occur. As with the analysis for NHI, there were significant genotypic differences for combined, rainfed, and irrigated treatments in 1999 but not in 2000 (Table 11). The genotype DOR 364 had a significantly higher ( $P \leq 0.001$ ) combined HI in 1999 than the genotypes ICA Palmar and 8-42-M-2 but not significantly higher than the other genotypes (Table 11). In both rainfed and irrigated treatments, the genotype ICA Palmar reported the lowest HI (Table 11) due to the fact that ICA Palmar did not mature or produce seed-N from most of the plots. Harvest index values are in agreement with those of Brunner and Zapata (1984) with faba bean (*Vicia faba minor*) and those obtained by Foster et al. (1995) with common bean. Harvest index values among the genotypes ranged from 6 to 41% in 1999 and from 25 to 40% in 2000 (Table 11) with DOR 364 (nod & nn), XAN 176, PR9603-22 having one of the highest HI in 1999.

Significant correlations occurred between HI and seed weight in both years of the

study (0.93\*\*\* and 0.86\*\*\*, for 1999 and 2000, respectively). These results suggests that HI might be an efficient tool for evaluating common bean lines for adaptation to water deficits. However, care must be taken, although an increased HI may contribute to the increased yield potential of a genotype it may not be the greatest contributor to that genotype yield improvement (Kumudini et al., 2001). Reports on the association between increased HI and seed yield in soybean have been found to be contradictory. Some reports suggest that there's no correlation between an increased HI and soybean yield (Schapaugh and Wilcox, 1980; Morrison et al., 1999; Shiraiwa and Hashikawa, 1995) and others suggest that theirs is (Frederick et al., 1991).

#### ***Nitrogen use efficiency (NUE)***

Nitrogen use efficiency (NUE) did not differ among irrigated and rainfed treatments in 1999 either as seed NUE or as total NUE. In 2000, seed-NUE was significantly greater ( $P \leq 0.10$ ) in the rainfed treatment and total-NUE was greater ( $P \leq 0.01$ ) in the irrigated treatment. In 1999, when NUE was calculated as total-NUE, the genotype DOR 364 (nn) had the highest ( $P \leq 0.001$ ) NUE of all the genotypes except PR9603-22 in the combined harvest. It was significantly higher ( $P \leq 0.05$ ) than all the other genotypes in the rainfed treatment and significantly higher ( $P \leq 0.01$ ) than XAN 176, BAT 477 (nn), and SEA5 in the irrigated treatment (Table 13).

When NUE was calculated as seed-NUE the genotype ICA Palmar was not included in the analyses. The genotype DOR 364 (nn) still recorded the highest NUE of all the genotypes for combined, rainfed, and irrigated treatments (Table 13). The genotype SEA5 consistently recorded one of the lowest total- and seed-NUE in 1999.

Significant genotypic differences were only detected in the rainfed treatment for both total-NUE ( $P \leq 0.10$ ) and seed-NUE ( $P \leq 0.05$ ) in 2000, with the genotype XAN 176 having the highest NUE in both total- and seed-NUE (Table 14). From these results, it seems as though the genotype XAN 176 appears to be the most efficient of the genotypes with regard to NUE.

### *Nitrogen fixation*

The N difference method was used to estimate  $N_2$  fixation in this study. Two reference crops, BAT 477 (nn) and DOR 364 (nn) were utilized. In 1999, when BAT 477 (nn) was used as the reference crop, there was no significant difference between the two treatments and virtually no fixation occurred (Table 15). However, when DOR 364 (nn) was used as the reference crop, greater fixation ( $P \leq 0.10$ ) occurred in the irrigated treatment. The same trend continued in 2000. When the genotype DOR 364 (nn) was used as the reference crop, significant differences occurred between the treatments but not when BAT 477 (nn) was used as the reference crop (Table 15). However, all N fixation values were exceedingly low.

There were significant genotypic differences in 1999 when both BAT 477 (nn) and DOR 364 (nn) were used as the reference crop whereas there was no significant difference among the genotypes when either BAT 477 (nn) or DOR 364 (nn) was used as the reference crop in 2000 (Table 16). In 1999, with both reference crops, the genotype DOR 364 (nod) had significantly higher N fixation ( $P \leq 0.05$ ) than the genotype ICA Palmar but not significantly higher than the other genotypes (Table 16). The amount of N-fixed in 1999 ranged from a low of  $-34.9 \text{ kg ha}^{-1}$  (ICA Palmar with BAT 477 (nn)) as

the reference crop) to a high of 19.9 kg ha<sup>-1</sup> (DOR 364 (nod) with DOR 364 (nn) as the reference crop). In 2000, for both reference crops the genotype DOR 364 (nod) had a numerically higher amount of N-fixed than the other genotypes although it was not statistically significant (Table 16). The amount of N-fixed in 2000 ranged from a low of -1.1 kg ha<sup>-1</sup> (PR9603-22 with BAT 477 (nn) as the reference crop) to a high of 5.3 kg ha<sup>-1</sup> (DOR 364 with DOR 364 (nn) as the reference crop) (Table 16).

The amount of N<sub>2</sub> fixed by common bean reported in the literature ranges from 3 to 57 kg ha<sup>-1</sup> (Wani and Lee, 1992; Peoples and Crasswell, 1992; Wani et al., 1995). Our results reported here are on the low end of this scale and may not be representative of the genotype's N<sub>2</sub> fixing ability. In 1999, plants were severely infected with *Cercospora* and common bacterial blight, contributing to a significant loss of leaf from the plant before seed filling which may have decreased photosynthates available for the nodules and for nitrogenase activity. In 2000, plots were located in the same location as the previous year. Plants exhibited *Cercospora*, common bacterial blight, and nitrogen deficiency symptoms. No starter fertilizer N was applied to the field in 1999 or 2000 in order to assess N fixation and soil-N status was low to medium. It is known that common bean is a poor N<sub>2</sub> fixer (Westermann et al., 1981) and it has been reported that a starter fertilizer N is usually required (Sprent and Thomas, 1984) to avoid periods of nitrogen stress in the field. In 2000, plants exhibited symptoms of zinc and/or iron deficiency. Iron deficiency would have interfered with nitrogenase activity helping to explain the low N fixation. Tissue analysis for zinc and iron is in progress.

Temperature may have also impacted the amount of N-fixed. Waters et al. (1983)



stated that satisfactory N<sub>2</sub> fixation may seldom be achieved in warm tropical locations. These authors found poor nodulation, lower N<sub>2</sub> fixation, and lower yields in a hot valley relative to a cool mountainous site (at mid-day, soil temperature at 10 cm averaged 28 and 20°C, respectively). Small and Joffe (1968) and Piha and Munns (1987) reported that N-fertilized legumes can better withstand warmer temperatures than can unfertilized counterparts such as the case with my study. The performance of the two reference crop BAT 477 (nn) and DOR 364 (nn) was unexpected. In both years of the study, N fixation was greater using DOR 364 (nn) than BAT 477 (nn).

### **Conclusion**

BAT 477 (nod) was one of the genotypes with the highest root-N concentration. XAN 176, DOR 364 (nn), and PR9603-22 had relatively high root-N concentration and were among the highest yielding lines in the field study. ICA Palmar had the highest stem-N concentration, probably resulting from its failure to mature and low N remobilization from stems to seeds. Leaf-N concentration was highest in 8-42-M-2 in 1999 and in DOR 364 (nn) in 2000. Nitrogen HI values among genotypes ranged from 7 to 76% which are comparable to values reported in the literature. XAN 176 had high NHI and HI in 1999 and high NUE in 2000. There were significant correlations between NHI and seed weight and between HI and seed weight suggesting that NHI and HI might be efficient tool for evaluating common bean lines for adaptation to water deficits. Nitrogen use efficiency did not differ among irrigated and rainfed treatments in 1999 but were greater in the irrigated treatment in 2000. Total N-fixed among the treatments (irrigated and rainfed) and among the genotypes was quite small and in some cases

negligible. N fixation was low and no fixation ( $-34.3 \text{ kg ha}^{-1}$ ) to  $19.9 \text{ kg ha}^{-1}$ . The reference crop DOR 364 (nn) gave a higher estimate of N fixation than did reference crop BAT 477 (nn). Genotypes varied for N-concentration of various organs, NUE, NHI, HI, and N fixation.

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Table 1. The effect of moisture stress on root, stem, leaf, and reproductive structures-N concentration (g kg<sup>-1</sup>) in common bean (*Phaseolus vulgaris* L.) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 1999 and 2000, under irrigated and rainfed moisture conditions and in polyvinyl chloride (PVC) tubes in a greenhouse at Michigan State University, East Lansing, MI. in 2000, under stressed and nonstressed moisture condition. N = 36 (UVI), N = 27 (PVC).

Treatments‡	1999			2000			PVC
	V3	R2	R7	V3	R4	R8	R2
	<b>g kg<sup>-1</sup></b>						
	<b>Root-N</b>						
Irrigated	12.5 ns§	8.0 ns	7.5 ns	10.3 ns	6.0 ns	8.6 ns	---
Rainfed	12.4	7.7	7.5	10.0	6.4	8.3	---
	<b>Stem-N</b>						
Irrigated	13.5 ns	9.9 ns	7.1 ns	13.6+	5.6 ns	7.7 ns	14.8 ns
Rainfed	11.9	9.9	6.6	14.3	6.7	8.1	14.8
	<b>Leaf-N</b>						
Irrigated	35.3 ns	23.2 ns	25.8*	30.6**	16.0+	17.0**	34.6 ns
Rainfed	35.6	20.0	21.9	32.7	19.4	19.0	35.7
	<b>Reproductive structures-N</b>						
Irrigated	---	¶37.6 ns	#17.2*	---	17.3*	††30.3 ns	30.2 ns
Rainfed	---	37.2	14.0	---	22.3	30.5	30.3
Irrigated	---	‡‡31.6 ns	§§34.4 ns	---	---	¶¶5.6 ns	---
Rainfed	---	30.8	35.4	---	---	5.9	---

‡ Indicate stressed and nonstressed moisture conditions in the PVC experiment (Irrigated = Nonstress; Rainfed = Stress).

§ Indicates no significant differences among means within a column.

¶ Indicate values from flowers within a column at R2 growth stage in 1999.

# Indicate values from pods within a column at R7 growth stage in 1999.

†† Indicate values from seeds within a column at R8 growth stage in 2000.

‡‡ Indicate values from pods within a column at R2 growth stage in 1999.

§§ Indicate values from seeds within a column at R7 growth stage in 1999.

¶¶ Indicate values from pods within a column at R8 growth stage in 2000.

\*\* , \* , +. Indicates significant difference among means within a column at P ≤ 0.01, 0.05, and 0.10, respectively, according to DMRT.

Table 2. Root-N concentration (g kg<sup>-1</sup>) of nine common bean (*Phaseolus vulgaris* L.) genotypes harvested at three growth stages (V3, R2, and R7) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 1999 under rainfed and irrigated moisture conditions. N = 8 (combined), 4 (rainfed or irrigated).

Genotypes	Combined			Rainfed			Irrigated		
	V3	R2	R7	V3	R2	R7	V3	R2	R7
BAT 477 (nod)	14.1 a*	8.90 a*	8.50 ab***	12.84 ns†	9.16 a*	8.26 ab**	15.42 a**	8.69 ns	8.75 a*
PR9603-22	12.0 abcd	7.00 b	7.00 bc	12.94	6.76 c	7.02 b	11.04 bc	7.14	6.97 bc
DOR 364 (nn)	11.7 bcd	6.80 b	6.30 c	12.41	6.67 c	6.19 b	10.89 bc	6.89	6.35 c
ICA Palmar	11.4 cd	8.90 a	9.30 a	11.27	8.66 abc	10.1 a	11.44 bc	9.12	8.43 ab
XAN 176	12.4 abcd	7.00 b	6.70 c	12.24	6.49 c	6.48 b	12.62 abc	7.57	6.96 bc
BAT 477 (nn)	10.7 d	7.40 ab	7.60 bc	11.26	6.90 bc	7.66 b	10.08 c	7.90	7.58 abc
SEAS	13.1 abc	8.40 ab	7.60 bc	13.05	9.02 ab	7.76 b	13.12 abc	7.84	7.36 abc
8-42-M-2	13.8 ab	8.40 ab	7.80 bc	13.38	8.63 abc	7.36 b	14.27 ab	8.25	8.23 ab
DOR 364 (nod)	12.9 abcd	7.60 ab	6.60 c	12.53	7.63 abc	6.76 b	13.24 abc	7.60	6.50 c
Mean	12.5	7.80	7.50	12.43	7.77	7.51	12.46	7.89	7.46

\*\*\*, \*\*, \*. Indicates significant difference among means within a column at P ≤ 0.001, 0.01, and 0.05, respectively, according to DMRT.

† indicates no significant differences among means within a column.



Table 3. Root-N concentration (g kg<sup>-1</sup>) of nine common bean (*Phaseolus vulgaris* L.) genotypes harvested at three growth stages (V3, R4, and R8) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 2000 under rainfed and irrigated moisture conditions. N = 8 (combined), 4 (rainfed or irrigated).

Genotypes	Combined			Rainfed			Irrigated		
	V3	R4	R8	V3	R4	R8	V3	R4	R8
BAT 477 (nod)	10.3 ns‡	5.70 ab+	7.70 ns	10.3 ns	6.84 a**	7.82 ns	10.3 ns	4.79 ns	8.59 ns
PR9603-22	10.3	5.30 ab	8.10	10.0	6.92 a	7.35	10.5	5.05	7.99
DOR 364 (nn)	10.5	7.30 ab	9.40	10.1	8.43 a	8.43	11.0	7.26	10.5
ICA Palmar	10.0	8.20 a	8.80	10.2	7.97 a	8.15	9.94	8.20	9.20
XAN 176	9.20	4.50 b	8.50	9.28	4.85 ab	8.23	9.07	4.89	8.50
BAT 477 (nn)	10.1	5.40 ab	7.60	8.85	5.92 ab	7.30	11.3	5.50	7.67
SEA5	1.00	4.80 b	9.10	11.0	1.95 b	8.49	11.0	7.77	9.01
8-42-M-2	10.4	5.40 ab	9.00	11.2	8.28 a	9.15	9.50	2.69	8.01
DOR 364 (nod)	9.60	7.20 ab	8.00	9.23	6.85 a	7.71	9.90	7.66	8.53
Mean	10.14	5.96	8.45	10.0	6.44	8.07	10.3	5.98	8.66

\*\*\*, \*\*, \*. Indicates significant difference among means within a column at P ≤ 0.001, 0.01, and 0.05, respectively, according to DMRT.

‡ Indicates no significant differences among means within a column.

Table 4. Stem-N concentration (g kg<sup>-1</sup>) of nine common bean (*Phaseolus vulgaris* L.) genotypes harvested at three growth stages (V3, R2, and R7) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 1999 under rainfed and irrigated moisture conditions. N = 8 (combined), 4 (rainfed or irrigated).

Genotypes	Combined			Rainfed			Irrigated		
	Growth stages								
	V3	R2	R7	V3	R2	R7	V3	R2	R7
BAT 477 (nod)	12.4 nst	10.0 ns	7.2 b***	11.1 ns	8.52 ns	6.60 bc***	13.7 ns	11.5 ns	7.79 ab*
PR9603-22	10.5	9.00	6.1 bc	8.75	9.43	5.98 bc	12.3	8.48	6.24 bc
DOR 364 (nn)	8.80	8.50	5.8 bc	9.58	8.09	5.68 bc	7.91	8.90	5.86 c
ICA Palmar	13.1	10.7	9.2 a	8.79	11.1	9.62 a	17.4	10.4	8.80 a
XAN 176	11.5	10.0	5.5 c	13.9	9.70	4.54 c	9.10	10.3	6.37 bc
BAT 477 (nn)	12.4	10.1	7.2 b	9.29	9.85	6.80 b	15.6	10.2	7.56 abc
SEA5	11.6	10.1	7.2 b	9.41	11.9	7.35 b	13.9	8.19	7.01 abc
8-42-M-2	15.7	11.0	7.3 b	20.2	10.4	6.55 bc	11.3	11.4	8.08 ab
DOR 364 (nod)	18.2	10.1	6.2 bc	16.1	10.3	6.12 bc	20.4	9.90	6.30 bc
Mean	12.7	9.92	6.7	11.9	9.92	6.58	13.5	9.92	7.11

† Indicates no significant differences among means within a column.

\*\*\*, \*. Indicates significant difference among means within a column at P ≤ 0.001 and 0.05, respectively, according to DMRT.

Table 5. Stem-N concentration (g kg<sup>-1</sup>) of nine common bean (*Phaseolus vulgaris* L.) genotypes harvested at three growth stages (V3, R4, and R8) grown at the Agricultural Experiment Station at the University of the Virgin Islands (UVI) in 2000 under rainfed and irrigated moisture conditions and in polyvinyl chloride (PVC) tubes in a greenhouse at Michigan State University, East Lansing, MI. in 2000, under stressed and nonstressed moisture conditions. N = (UVI)8 (combined), 4 (rainfed or irrigated); (PVC) N = 6 (combined), 3 (stress or nonstress).

Genotypes	UVI									PVC				
	Combined			Rainfed			Irrigated			Combined			Stress	Nonstress
	V3	R4	R8	V3	R4	R8	V3	R2	R8	R2	R2	R2	R2	R2
BAT 477 (nod)	13.0 ns†	5.80ns	8.40 ns	14.2 ns	6.60 ns	7.76 ns	13.3 ns	5.98 ns	8.50 b+	15.2 ab**	16.9 ns	13.5 abc*		
PR9603-22	13.7	5.30	7.20	13.5	6.42	7.08	13.0	4.12	6.70 b	12.3 b	12.4	12.2 c		
DOR 364 (nn)	14.1	7.00	7.80	15.0	7.48	7.31	13.2	6.46	8.00 b	15.7 a	15.1	16.3 a		
ICA Palmar	14.7	9.20	9.70	14.3	8.39	6.67	13.3	8.74	12.1 a	17.5 a	18.5	16.5 a		
XAN 176	14.7	5.10	7.80	14.2	5.55	8.80	15.2	4.14	7.44 b	14.6 ab	15.0	14.2 abc		
BAT 477 (nn)	13.9	6.50	7.40	13.6	7.31	8.20	13.5	4.94	6.16 b	15.1 ab	14.7	15.4 ab		
SEAS	14.0	4.60	8.00	13.8	2.53	8.65	14.3	6.50	7.75 b	16.5 a	16.6	16.4 a		
8-42-M-2	14.1	5.30	7.20	10.6	6.88	7.96	13.4	2.59	5.87 b	14.6 ab	16.0	13.1 bc		
DOR 364 (nod)	14.0	6.40	8.00	13.6	7.47	7.67	13.5	7.02	7.16 b	15.4 ab	15.0	16.0 ab		
Mean	14.0	6.13	7.93	13.6	6.51	7.79	13.6	5.61	7.74	15.2	15.6	14.8		

† Indicates no significant differences among means within a column.

\*\* , \* , +. Indicates significant difference among means within a column at P ≤ 0.01, 0.05, and 0.10, respectively, according to DMRT.

Table 6. Leaf-N concentration ( $\text{g kg}^{-1}$ ) of nine common bean (*Phaseolus vulgaris* L.) genotypes harvested at three growth stages (V3, R2, and R7) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 1999 under rainfed and irrigated moisture conditions. N = 8 (combined), 4 (rainfed or irrigated).

Genotypes	Combined						Rainfed						Irrigated					
	V3		R2		R7		V3		R2		R7		V3		R2		R7	
	Growth stages																	
	$\text{g kg}^{-1}$																	
BAT 477 (nod)	34.8 ns†	19.3 bc*	24.1 abcd***	37.2 ns	13.1 c+	23.8 abc**	32.4 bc+	24.1 ab+	24.4 abc*	37.9	25.2 ab	19.1 cd	37.9	24.3 abc	16.2 c	37.9 ab	31.0 a	22.0 bc
DOR 364 (nn)	36.1	15.0 c	18.4 d	37.3	15.6 c	18.0 bc	34.8 abc	16.9 b	18.9 c	37.4	16.5 bc	28.3 a	40.1	11.0 c	29.0 a	34.8 abc	20.2 b	27.7 ab
XAN 176	36.1	20.9 abc	23.2 abcd	33.2	19.4 abc	19.3 bc	39.1 a	24.0 ab	27.2 ab	33.0	22.1 abc	26.7 ab	33.4	26.2 abc	24.1 abc	32.6 bc	19.2 b	29.4 ab
SEA5	35.4	18.7 bc	25.2 abc	30.6	16.7 bc	23.0 abc	40.2 a	20.2 b	27.5 ab	31.6	30.3 a	28.0 a	33.0	32.5 ab	24.7 ab	30.2 c	30.2 a	31.2 a
DOR 364 (nod)	36.6	26.3 ab	21.2 bcd	37.4	33.7 a	18.8 bc	35.7 abc	22.8 ab	23.6 abc	35.4	21.6	23.8	35.6	21.4	21.9	35.3	23.2	25.8
Mean	35.4	21.6	23.8	35.6	21.4	21.9	35.3	23.2	25.8									

† Indicates no significant differences among means within a column.

\*\*\*, \*\*, \*, +. Indicates significant difference among means within a column at  $P \leq 0.001$ , 0.01, 0.05, and 0.10, respectively, according to DMRT.

Table 7. Leaf-N concentration ( $\text{g kg}^{-1}$ ) of nine common bean (*Phaseolus vulgaris* L.) genotypes harvested at three growth stages (V3, R4, and R8) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 2000 under rainfed and irrigated moisture conditions and in polyvinyl chloride (PVC) tubes in a greenhouse at Michigan State University, East Lansing, MI. in 2000, under stressed and nonstressed moisture conditions. N = (UVI) 8 (combined), 4 (rainfed or irrigated); (PVC) N = 6 (combined), 3 (stress or nonstress).

Genotypes	UVI												PVC		
	Combined			Rainfed			Irrigated			Combined			Stress	Nonst.	
	V3	R4	R8	V3	R4	R8	V3	R4	R8	V3	R4	R8	R2	R2	R2
BAT 477 (nod)	32.4 ab*	17.9ns†	18.5ns	31.8 bc*	20.5 ns	18.5 ns	33.1 ab*	15.3 ns	18.1 ns	18.1 ns	34.4 ns	33.4	c+	35.4 ns	
PR9603-22	31.1 b	16.2	18.5	29.3 c	19.0	18.1	32.9 ab	13.4	17.7	17.7	33.2	32.8	c	33.6	
DOR 364 (nn)	34.3 a	19.2	19.1	35.8 a	18.4	18.3	32.8 ab	20.0	17.2	17.2	37.8	41.1 a		34.7	
ICA Palmar	32.3 ab	23.3	18.7	33.7 ab	25.7	17.6	31.0 abc	21.0	17.1	17.1	35.2	35.6 bc		34.8	
XAN 176	30.7 b	14.8	18.8	32.5 abc	17.7	20.4	28.9 bc	11.8	18.1	18.1	37.8	39.0 ab		36.7	
BAT 477 (nn)	30.2 b	17.3	19.3	31.0 bc	19.3	20.8	30.0 abc	15.3	18.4	18.4	34.4	36.0 bc		32.8	
SEA5	33.3 ab	12.8	18.0	32.4 abc	6.66	18.0	34.2 a	19.0	15.7	15.7	34.0	33.1 c		34.9	
8-42-M-2	30.6 b	16.5	17.6	34.2 ab	25.2	20.2	27.0 c	7.76	16.2	16.2	35.0	36.3 bc		33.7	
DOR 364 (nod)	30.6 b	21.1	19.1	32.3 abc	22.4	19.5	28.8 bc	19.9	17.8	17.8	34.6	34.4 bc		34.8	
Mean	31.7	17.7	18.6	32.5	19.4	19.0	30.9	15.9	17.4	17.4	35.2	35.7		34.6	

†, ‡, +. Indicates no significant differences among means within a column.

†, +. Indicates significant difference among means within a column at  $P \leq 0.05$  and  $0.10$ , respectively, according to DMRT.

Table 8a. Reproductive structures-N concentration ( $\text{g kg}^{-1}$ ) of nine common bean (*Phaseolus vulgaris* L.) genotypes harvested at three growth stages (V3, R2, and R7) grown at the Agricultural Experiment Station at the University of the Virgin Islands in 1999 under rainfed and irrigated moisture conditions. N = 8 (combined), 4 (rainfed or irrigated).

Genotypes	-----Combined-----			
	-----Growth stages-----			
	R2 (flowers)	R2 (pods & seeds)	R7 (pods)	R7 (seeds)
	----- $\text{g kg}^{-1}$ -----			
BAT 477 (nod)	38.8 abc+	34.5 b***	18.3 abc***	38.6 ab**
PR9603-22	36.1 bcd	31.1 bc	10.9 cd	32.7 bc
DOR 364 (nn)	34.5 d	28.1 cd	7.50 d	30.2 c
ICA Palmar	40.0 a	34.4 b	23.4 a	32.7 bc
XAN 176	38.5 abc	22.3 e	13.9 bcd	36.3 ab
BAT 477 (nn)	39.6 ab	34.0 b	14.5 abcd	36.0 abc
SEA5	35.5 cd	33.8 b	21.5 ab	39.0 a
8-42-M-2	38.7 abc	24.7 de	22.0 ab	35.6 abc
DOR 364 (nod)	38.4 abc	38.7 a	8.80 d	33.5 abc
Mean	37.8	31.3	15.6	34.9
	-----Rainfed-----			
BAT 477 (nod)	36.9 ns‡	33.4 ab***	13.6 ab**	37.9 ab*
PR9603-22	35.7	31.2 abc	6.50 b	33.2 bc
DOR 364 (nn)	35.3	27.3 bcd	6.91 b	30.8 c
ICA Palmar	40.0	34.8 a	25.1 a	33.1 bc
XAN 176	38.9	21.7 d	8.28 b	34.6 abc
BAT 477 (nn)	40.4	33.5 ab	17.6 ab	38.8 ab
SEA5	33.4	32.6 ab	23.8 a	40.2 a
8-42-M-2	39.1	25.0 cd	17.3 ab	36.5 abc
DOR 364 (nod)	39.1	37.7 a	7.02 b	33.5 bc
Mean	37.6	30.8	14.0	35.4

Table 8b.

Genotypes	-----Irrigated-----			
	-----Growth stages-----			
	R2 (flowers)	R2 (pods & seeds)	R7 (pods)	R7 (seeds)
	-----g kg <sup>-1</sup> -----			
BAT 477 (nod)	38.4 abc*	35.6 ab***	22.9 ab*	39.3 a+
PR9603-22	33.0 c	31.0 bc	15.3 bcd	32.3 bc
DOR 364 (nn)	35.0 bc	28.9 c	8.13 d	29.6 c
ICA Palmar	36.7 abc	34.0 b	21.7 abc	32.3 bc
XAN 176	38.0 abc	22.8 d	19.4 abc	37.9 ab
BAT 477 (nn)	38.1 abc	34.3 b	11.4 cd	32.8 bc
SEA5	40.0 ab	35.0 b	19.3 abc	37.8 ab
8-42-M-2	41.6 a	24.5 d	26.5 a	34.7 abc
DOR 364 (nod)	34.6 bc	39.7 a	10.6 cd	33.4 bc
Mean	37.3	31.8	17.2	34.4

\*\*\*, \*\*, \*, +. Indicates significant difference among means within a column at  $P \leq 0.001$ , 0.01, 0.05, and 0.10, respectively, according to DMRT.

‡ Indicates no significant differences among means within a column.

Table 9. Reproductive structures-N concentration ( $\text{g kg}^{-1}$ ) of nine common bean (*Phaseolus vulgaris* L.) genotypes harvested at three growth stages (V3, R4, and R8) grown at the Agricultural Experiment Station at the University of the Virgin Islands (UVI) in 2000 under rainfed and irrigated and irrigated moisture conditions and in polyvinyl chloride (PVC) tubes in a greenhouse at Michigan State University, East Lansing, MI. in 2000, under stressed and nonstressed moisture conditions. N = (UVI) 8 (combined), 4 (rainfed or irrigated); (PVC) N = 6 (combined), 3 (stress or nonstress).

Genotypes	PVC												
	UVI				PVC				PVC				
	Combined		Rainfed		Irrigated		Nonstress		Stress		Nonstress		
	R4	R8 seeds	R8 pods	R4	R8 seeds	R8 pods	R4	R8 seeds	R8 pods	R2	R2	R2	R2
$\text{g kg}^{-1}$													
	Growth stages												
BAT 477 (nod)	20.0 ns†	32.7 ns	6.20 ns	25.1 ns	32.9 ab*	4.6 ns	15.0 ns	32.6 ns	7.59 ns	30.6	cde***	29.2 bc**	32.0 abc**
PR9603-22	17.9	28.5	4.80	22.2	28.3 abc	5.2	13.6	28.8	5.02	26.8	e	24.4 c	29.2 bc
DOR 364 (nn)	24.1	32.4	5.30	25.3	30.5 abc	5.1	22.8	34.3	5.65	32.8	bcd	33.2 b	32.4 abc
ICA Palmar	24.2	27.0	5.00	32.2	30.8 abc	5.0	16.2	23.2	5.13	†	---	---	---
XAN 176	16.6	29.3	5.50	17.3	25.6 c	6.8	16.0	33.1	4.36	42.9 a		45.1 a	40.6 a
BAT 477 (nn)	19.2	31.0	6.80	20.6	32.4 ab	8.6	17.7	29.5	5.02	37.1 b		35.6 b	38.5 ab
SEA5	15.0	28.4	5.10	8.46	26.9 bc	5.4	21.4	30.0	5.11	28.7	de	30.7 bc	26.8 c
8-42-M-2	18.4	31.4	6.30	27.0	32.3 ab	7.8	9.89	30.5	4.70	36.2	bc	36.8 ab	35.6 abc
DOR 364 (nod)	22.5	32.5	6.60	22.5	34.4 a	5.1	22.4	30.5	7.87	37.3 b		38.0 ab	36.7 abc
Mean	19.8	30.4	5.73	22.3	30.5	6.0	17.2	30.3	5.61	30.3		30.3	30.2

† Indicates no significant differences among means within a column.

\*\*\*, \*\*, \* Indicates significant difference among means within a column at  $P \leq 0.001, 0.01, \text{ and } 0.05$ , respectively, according to DMRT.

† Indicates no reproductive structures present.



Table 10. Nitrogen harvest index (NHI) of common bean (*Phaseolus vulgaris* L.) plants grown under irrigated (nonstressed) and rainfed (stressed) moisture regime in a 1999 and 2000 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. The NHI was computed as grams seed-N / grams total-N. N = 8 (combined), 4 (rainfed or irrigated).

Genotypes	1999				2000			
	Combined NHI	Rainfed NHI	Irrigated NHI	NHI (g seed N / g total N)	Combined NHI	Rainfed NHI	Irrigated NHI	Irrigated NHI
BAT 477 (nod)	0.43 abc***	0.51 abc***	0.35 abc**	0.61 ns†	0.61 ns†	0.74 ns	0.50 ns	0.50 ns
PR9603-22	0.62 ab	0.76 a	0.48 abc	0.63	0.63	0.61	0.69	0.69
DOR 364 (nn)	0.62 ab	0.67 ab	0.58 ab	0.59	0.59	0.71	0.50	0.50
ICA Palmar	0.10 d	0.07 d	0.12 c	0.45	0.45	0.64	0.30	0.30
XAN 176	0.60 ab	0.76 a	0.45 abc	0.63	0.63	0.53	0.75	0.75
BAT 477 (nn)	0.46 abc	0.42 bc	0.49 abc	0.57	0.57	0.43	0.73	0.73
SEAS	0.40 bc	0.35 c	0.45 abc	0.68	0.68	0.67	0.73	0.73
8-42-M-2	0.32 c	0.47 abc	0.18 bc	0.62	0.62	0.59	0.68	0.68
DOR 364 (nod)	0.66 a	0.69 ab	0.62 a	0.64	0.64	0.72	0.58	0.58
Mean	0.47	0.52	0.41	0.60	0.60	0.63	0.61	0.61

\*\*\*, \*\*. Indicates significant difference among means within a column at  $P \leq 0.001$  and  $0.01$ , respectively, according to DMRT.

† Indicates no significant differences among means within a column.

Table 11. Harvest index (HI) of nine common bean (*Phaseolus vulgaris* L.) plants grown under irrigated and rainfed moisture regime in a 1999 and 2000 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. The HI was as gram seed DW / gram total DW. N = 8 (combined), 4 (rainfed or irrigated).

Genotypes	1999			2000		
	Combined HI	Rainfed HI	Irrigated HI	Combined HI	Rainfed HI	Irrigated HI
BAT 477 (nod)	0.25 ab***	0.31 ab**	0.19 abc*	0.35 ns†	0.44 ns	0.26 ns
PR9603-22	0.38 a	0.47 a	0.29 ab	0.38	0.38	0.39
DOR 364 (nn)	0.37 a	0.40 ab	0.35 a	0.35	0.44	0.26
ICA Palmar	0.06 c	0.05 c	0.07 c	0.27	0.37	0.17
XAN 176	0.38 a	0.48 a	0.28 abc	0.38	0.34	0.42
BAT 477 (nn)	0.29 ab	0.24 bc	0.34 ab	0.34	0.25	0.43
SEA5	0.26 ab	0.22 bc	0.30 ab	0.42	0.43	0.42
8-42-M-2	0.20 bc	0.29 ab	0.12 bc	0.35	0.35	0.35
DOR 364 (nod)	0.41 a	0.42 ab	0.41 a	0.39	0.45	0.33
Mean	0.29	0.32	0.26	0.36	0.38	0.33

\*\*\*, \*\*, \*. Indicates significant difference among means within a column at P ≤ 0.001, 0.01, and 0.05, respectively, according to DMRT.

† Indicates no significant differences among means within a column.

Table 12. Nitrogen use efficiency (NUE) of common bean (*Phaseolus vulgaris* L.) plants grown under irrigated and rainfed moisture regime in a 1999 and 2000 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. The NUE was computed as total g DW / total g N or g seed DW / g seed N. N = 36.

Treatment	<u>NUE</u>	
	<u>g seed DW / g seed N</u>	<u>Total g DW / Total g N</u>
		<b>1999</b>
Irrigated	28 ns‡	49 ns
Rainfed	29	49
		<b><u>2000</u></b>
Irrigated	32+	63**
Rainfed	34	58

\*\* , +. Indicates significant difference among means within a column at  $P \leq 0.01$  and 0.10, respective, according to DMRT.

‡ Indicates no significant differences among means within a column.

Table 13. Nitrogen use efficiency (NUE) of nine common bean (*Phaseolus vulgaris* L.) plants grown under irrigated and rainfed moisture regime in a 1999 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. The NUE was computed as total g DW / total g N or g seed DW / g seed N. g seed DW / g seed N. N = 8 (combined), 4 (rainfed and irrigated).

Genotypes	-----Total NUE (total g DW / total g N)-----			-----Seed NUE (g seed DW / g seed N)-----		
	Combined NUE	Rainfed NUE	Irrigated NUE	Combined NUE	Rainfed NUE	Irrigated NUE
BAT 477 (nod)	46 bc***	45 b*	48 abc**	26 cd*	27 cde*	26 d+
PR9603-22	53 ab	50 b	55 ab	31 ab	31 ab	32 ab
DOR 364 (nn)	57 a	56 a	58 a	33 a	33 a	34 a
ICA Palmar	50 bc	48 b	51 abc	†--	---	---
XAN 176	46 bc	47 b	46 bc	28 bcd	29 bcd	27 cd
BAT 477 (nn)	46 bc	47 b	46 bc	28 bcd	26 de	31 ab
SEA5	44 c	46 b	43 c	26 d	25 e	27 cd
8-42-M-2	48 bc	48 b	47 abc	25 bcd	28 bcde	22 bcd
DOR 364 (nod)	49 bc	50 b	48 abc	30 abc	30 abc	30 bc
Mean	49	49	49	29	28	29

† ICA Palmar was not included in the analyses for seed NUE.

\*\*\*, \*\*, \*, †. Indicates significant difference among means within a column at P ≤ 0.001, 0.01, 0.05, and 0.10, respectively, according to DMRT.

Table 14. Nitrogen use efficiency (NUE) of nine common bean (*Phaseolus vulgaris* L.) plants grown under irrigated and rainfed moisture regime in a 2000 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. The NUE was computed as total g DW / total g N or g seed DW / g seed N. g seed DW / g seed N. N = 8 (combined), 4 (rainfed and irrigated).

Genotypes	Total NUE (total g DW / total g N)			Seed NUE (g seed DW / g seed N)		
	Combined NUE	Rainfed NUE	Irrigated NUE	Combined NUE	Rainfed NUE	Irrigated NUE
BAT 477 (nod)	58 ns‡	53 bc+	64 ns	31 ns	31 bc*	31 ns
PR9603-22	64	62 ab	66	35	35 abc	35
DOR 364 (nn)	60	55 abc	65	31	33 bc	29
ICA Palmar	61	59 ab	63	§	---	---
XAN 176	60	63 a	57	35	40 a	30
BAT 477 (nn)	63	65 a	60	33	31 bc	34
SEA5	60	60 ab	59	36	38 ab	34
8-42-M-2	62	55 abc	68	32	31 bc	33
DOR 364 (nod)	55	49 c	61	31	29 c	33
Mean	60	58	63	33	34	32

‡ Indicates no significant differences among means within a column.

\* , +. Indicates significant difference among means within a column at  $P \leq 0.05$  and  $0.10$ , respectively, according to DMRT.

§ ICA Palmar was not included in the analyses for seed NUE.

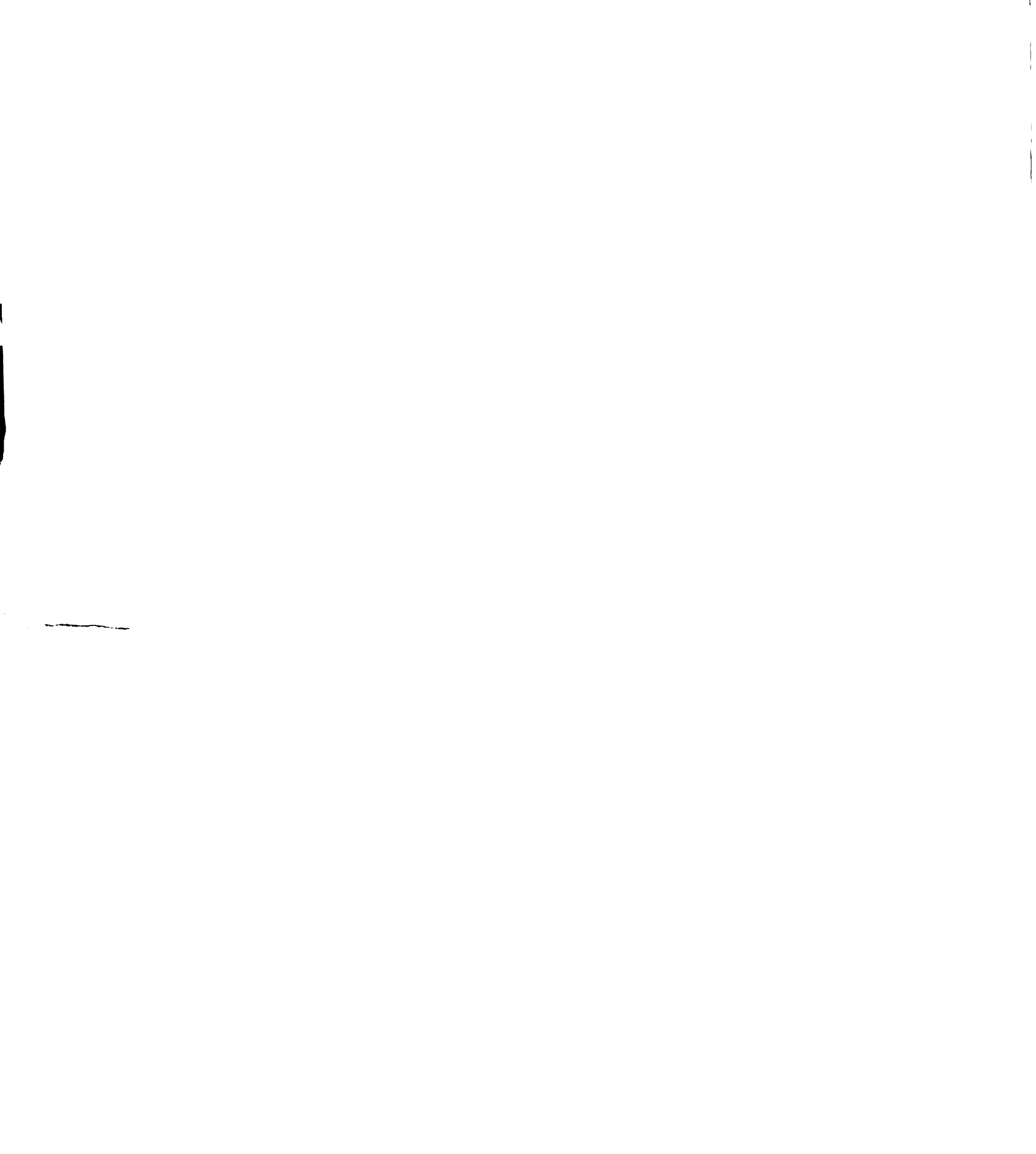


Table 15. Nitrogen fixed ( $\text{kg ha}^{-1}$ ) from common bean (*Phaseolus vulgaris* L.) plants grown under irrigated and rainfed moisture regime in a 1999 and 2000 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. N-fixed was calculated by the difference method with DOR 364 (nn) and BAT 477 (nn) as the reference crops. N = 36.

Treatment	-----N-fixed ( $\text{kg ha}^{-1}$ )-----	
	BAT 477 (nn)	DOR 364 (nn)
		<b>1999</b>
Irrigated	-16.2 ns‡	9.69+
Rainfed	-6.13	-1.4
		<b>2000</b>
Irrigated	0.09 ns	4.34**
Rainfed	1.34	-0.1

‡ Indicates no significant differences among means within a column.

\*\* , +. Indicates significant difference among means within a column at  $P \leq 0.01$  and 0.10, respectively, according to DMRT.

Table 16. Nitrogen fixed (kg ha<sup>-1</sup>) of nine common bean (*Phaseolus vulgaris* L.)

genotypes grown under irrigated and rainfed moisture regime in a 1999 and 2000 field study at the Agricultural Experiment Station at the University of the Virgin Islands-St. Croix campus. N-fixed was calculated by the difference method with DOR 364 (nn) and BAT 477 (nn) as the reference crops. N = 8

Genotypes	1999		2000	
	BAT 477 (nn)	DOR 364 (nn)	BAT 477(nn)	DOR 364(nn)
BAT 477 (nod)	-11.2 ab*	4.15 ab*	2.67 ns†	4.9 ns
PR9603-22	-11.3 ab	3.96 ab	-1.1	0.3
ICA Palmar	-34.9 b	-19.6 b	-0.8	0.6
XAN 176	0.10 a	15.4 a	-0.1	1.4
SEA5	-3.15 a	12.1 a	-0.4	1.0
8-42-M-2	-22.0 ab	-6.7 ab	0.80	2.2
DOR 364 (nod)	4.37 a	19.7 a	3.90	5.3
Mean	-11.2	4.12	0.71	2.25

\* Indicates significant difference among means within a column at  $P \leq 0.05$  according to DMRT.

† Indicates no significant differences among means within a column.



## Summary and Conclusions

The objectives of this study were to utilize limiting and non-limiting moisture regimes to determine (i) if selected genotypes of common beans exhibited differences in drought resistance as measured by yield, (ii) if drought resistance genotypes had differing root growth, and (iii) if genotypes differed in N fixation. The hypotheses were: (i) drought resistant genotypes have a greater mass of fine roots than drought susceptible genotypes and (ii) drought resistance genotypes have a higher N<sub>2</sub> fixing capacity than susceptible genotypes.

Geometric mean and the stress tolerance index (STI) gave identical rankings with regard to genotypic drought resistance and correlated more highly with yield than did the drought susceptibility index (DSI). Geometric mean and STI ranked PR9603-22, DOR 364 (nod), and DOR 364 (nn) among the top four genotypes in both 1999 and 2000. Abscisic acid (ABA) increased total root length (TRL) and moisture stress decreased it. Nitrogen fixation was low both years, ranging from no fixation (-34.3 kg ha<sup>-1</sup>) to 19.9 kg ha<sup>-1</sup>, possibly reflecting iron deficiency and problems resulting from infestations of common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*), *Cercospora* (*Cercospora canescens*). Thus genotypic differences in N fixation could not be determined.

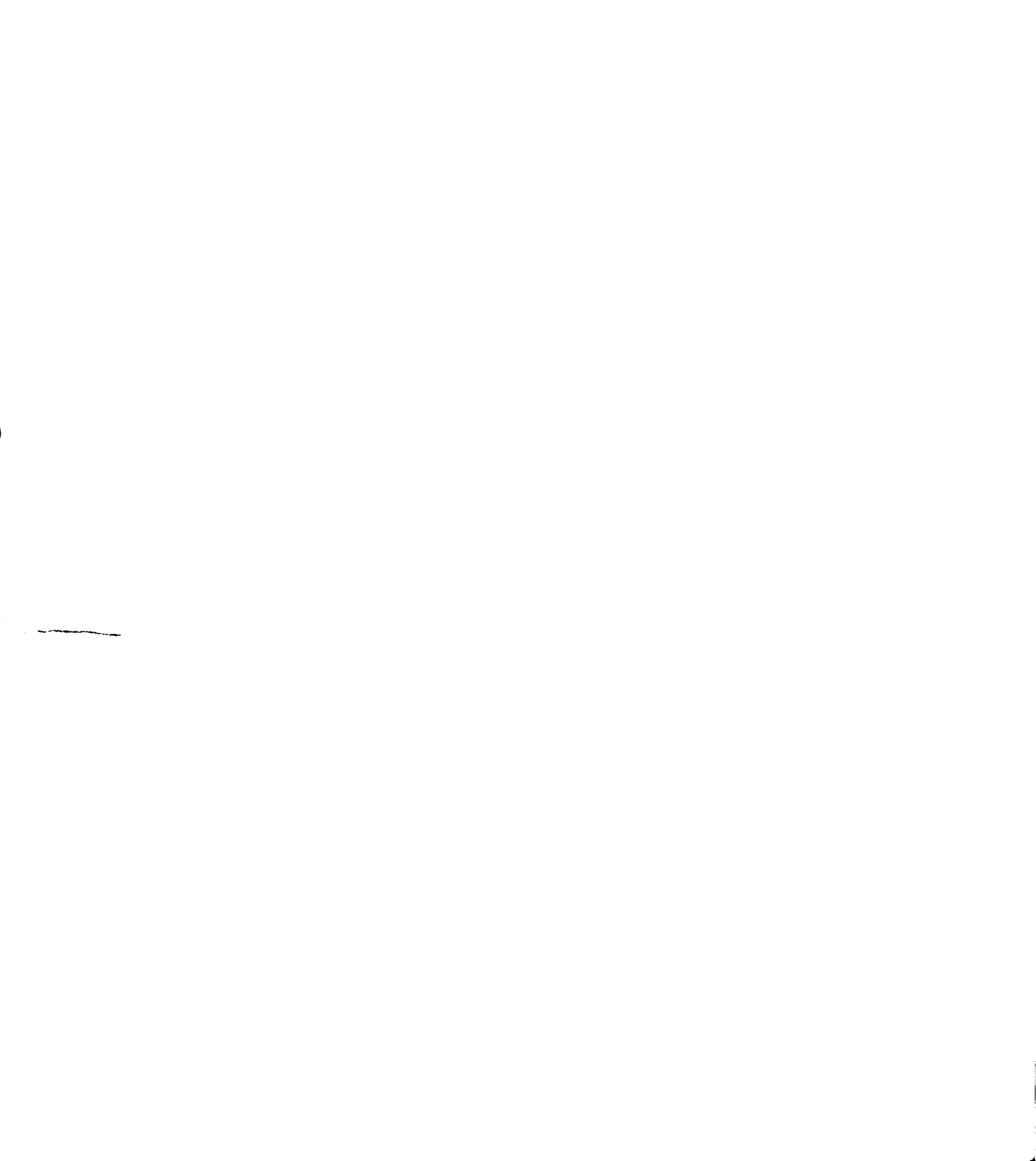
Genotypes varied for yield, TRL, NUE, NHI, and N fixation. Growth pouch and PVC studies identified XAN 176, SEA5, and 8-42-M-2 as having high TRL, suggesting that growth pouches may provide a viable method for assessing root growth among differing lines. Fine roots made the largest contribution to total root length, suggesting

that water absorption may be more associated with fine than large roots. The susceptible check (8-42-M-2), produced a greater root length and a greater mass of fine roots than the resistant check BAT 477 [nodulating (nod)] suggesting that the mechanism that help imparts drought resistance in the resistant check may not be associated with root length and/or the accumulation of finer roots.

Overall genotypic rankings were generated using the genotypic rankings from (i) 1999 and 2000 yield data, (ii) TRL from the ABA and control growth pouch and the polyvinyl chloride (PVC) studies, (iii) 1999 and 2000 geometric mean, and (iv) 1999 and 2000 N fixation using DOR 364 (nn) as the reference crop (Appendix B). A ranking of one was the highest and eight the lowest. Individual genotypic rankings were tallied and the genotype with the lowest number was assigned a ranking of one and the genotype with the highest number a ranking of eight. To my knowledge, a categorization of this type has not been presented before. The overall ranking was created by giving equal weight to each of the individual rankings. Future work may indicate otherwise, but the proposed system is one method for processing the data to assess relative genotypic performance. To that end, the three highest performers were DOR 364 (nod), XAN 176, and PR9603-22.

Appendix A. Weather watch 1999 processed daily outputs from 9 March to 1 June, 1999 for the Agricultural Experiment Station at the University of the Virgin Islands, USVI..

Month	Day	†AAT	‡MxAT	§MnAT	¶Avg. Str.	#Pot. ET	†Mx WS	‡‡Avg WS	§§Tot. rain	¶¶Mx Soil T	#Mn Soil T
3	9	73.70	80.5	67.95	0.18	0.107	18.0	4.4	0.00	74.3	71.1
3	10	74.50	71.7	67.14	0.29	0.158	17.2	5.0	0.00	75.4	71.4
3	11	74.20	82.2	65.53	0.25	0.131	17.7	3.4	0.00	77.0	72.4
3	12	75.50	82.0	67.12	0.31	0.156	17.5	3.4	0.00	78.8	73.8
3	13	74.20	83.6	65.30	0.26	0.132	15.2	2.7	0.00	79.8	73.9
3	14	76.20	83.0	70.50	0.28	0.147	17.7	5.2	0.00	79.6	74.4
3	15	77.20	82.2	73.90	0.27	0.147	19.7	8.4	0.00	79.9	75.4
3	16	77.00	82.7	72.00	0.29	0.152	18.5	4.2	0.00	81.0	76.5
3	17	74.20	83.5	64.58	0.26	0.135	17.5	3.4	0.00	81.1	75.2
3	18	76.90	83.6	71.60	0.22	0.131	22.4	6.1	0.00	79.7	75.4
3	19	77.50	83.5	73.60	0.24	0.148	20.0	7.0	0.00	78.5	75.1
3	20	77.30	83.1	72.30	0.26	0.149	18.5	4.9	0.00	80.0	75.7
3	21	76.10	83.0	70.90	0.17	0.108	19.8	4.6	0.00	79.4	75.4
3	22	77.20	83.0	72.80	0.26	0.150	22.5	6.8	0.00	78.4	75.1
3	23	77.10	83.1	71.90	0.23	0.122	18.2	5.8	0.04	81.0	77.4
3	24	77.40	83.2	72.20	0.26	0.136	19.2	5.0	0.03	80.2	77.1
3	25	77.30	83.3	72.90	0.23	0.122	20.0	4.4	0.03	82.3	77.4
3	26	79.10	84.0	75.60	0.28	0.149	21.7	8.4	0.00	80.6	77.0
3	27	79.20	84.0	76.30	0.31	0.170	22.7	9.3	0.00	82.2	77.7



3	3	28	78.20	84.0	76.00	0.28	0.155	23.9	8.1	0.00	83.1	78.5
3	3	29	77.30	83.7	74.50	0.21	0.125	20.2	6.5	0.00	82.6	77.9
3	3	30	77.50	84.7	72.50	0.21	0.123	19.3	6.0	0.03	80.6	76.4
3	3	31	78.00	84.5	73.20	0.30	0.169	20.9	7.2	0.00	81.0	76.2
4	4	1	78.00	84.2	75.10	0.20	0.122	22.4	9.0	0.00	81.4	76.2
4	4	2	78.30	83.6	74.30	0.28	0.158	20.5	7.6	0.00	79.6	75.6
4	4	3	76.00	83.8	68.73	0.20	0.094	17.2	2.5	0.68	80.4	75.4
4	4	4	76.20	82.0	71.40	0.21	0.103	18.3	2.4	0.05	78.4	74.9
4	4	10	73.0	80.3	69.23	0.08	0.035	12.5	1.5	1.57	71.7	69.89
4	4	11	75.0	81.8	68.35	0.17	0.074	10.3	1.4	0.00	73.5	70.5
4	4	12	75.7	82.6	68.53	0.26	0.123	14.1	2.0	0.00	74.6	71.4
4	4	13	75.6	84.0	68.32	0.29	0.139	13.6	1.8	0.00	77.4	72.9
4	4	14	76.9	84.2	70.40	0.30	0.152	13.8	2.4	0.00	79.1	74.7
4	4	15	79.0	86.1	72.70	0.26	0.141	17.8	3.3	0.00	80.8	76.6
4	4	16	80.3	87.3	75.50	0.29	0.164	20.2	6.5	0.00	81.3	77.1
4	4	17	80.7	87.6	76.10	0.23	0.148	22.0	8.0	0.00	82.0	77.5
4	4	18	81.0	87.8	76.60	0.23	0.141	20.9	8.6	0.00	80.5	76.3
4	4	19	80.3	86.9	75.40	0.19	0.125	22.9	9.9	0.02	80.3	76.5
4	4	20	78.8	83.6	73.40	0.25	0.140	25.0	7.3	0.26	78.4	75.3
4	4	21	80.3	85.1	77.10	0.31	0.172	21.7	9.7	0.00	80.5	76.7
4	4	22	79.5	84.5	75.10	0.24	0.146	23.4	9.1	0.00	81.2	76.6
4	4	23	78.9	84.1	74.70	0.19	0.121	21.2	8.3	0.00	79.3	75.8
4	4	24	78.4	82.8	72.90	0.17	0.099	20.7	6.5	0.39	78.6	75.8

4	4	25	75.9	83.5	69.34	0.15	0.074	14.0	2.0	0.22	79.4	75.4
4	4	26	76.0	84.0	69.16	0.19	0.093	14.1	2.0	0.14	78.0	74.7
4	4	27	78.0	83.8	73.30	0.15	0.084	17.5	3.0	0.06	77.8	75.3
4	4	28	79.0	84.1	73.50	0.23	0.118	18.8	3.7	0.00	78.7	76.0
4	4	29	80.6	85.1	77.40	0.31	0.160	18.2	6.2	0.00	80.4	77.4
4	4	30	80.4	83.8	77.70	0.30	0.163	17.3	6.5	0.00	82.0	77.9
5	5	1	79.9	84.5	73.80	0.31	0.162	18.0	5.0	0.00	81.9	78.0
5	5	2	81.3	85.0	78.30	0.31	0.162	19.7	5.8	0.00	82.5	78.8
5	5	3	81.1	85.5	76.60	0.29	0.151	19.3	6.1	0.01	82.8	79.3
5	5	4	80.8	85.1	76.80	0.31	0.167	18.8	5.2	0.00	82.9	79.2
5	5	5	80.2	85.1	76.20	0.30	0.161	17.8	4.0	0.01	85.7	80.6
5	5	6	79.9	84.8	73.60	0.29	0.158	17.5	3.8	0.00	84.1	80.1
5	5	7	79.5	84.4	74.10	0.30	0.163	15.5	3.5	0.00	83.1	79.3
5	5	8	78.4	85.7	70.90	0.22	0.104	12.0	1.2	0.00	82.7	78.6
5	5	9	78.6	85.5	72.60	0.24	0.130	15.8	3.3	0.13	80.5	77.7
5	5	10	78.3	84.3	72.80	0.30	0.164	16.3	3.7	0.00	80.8	77.4
5	5	11	78.2	84.2	75.3	0.13	0.064	14.5	2.2	0.06	80.7	77.7
5	5	12	77.4	82.9	74.0	0.12	0.044	11.8	0.9	0.27	78.3	76.6
5	5	13	81.4	86.0	78.3	0.26	0.141	17.0	5.6	0.00	78.7	77.3
5	5	14	82.0	86.4	78.3	0.30	0.160	19.0	4.7	0.00	80.2	78.3
5	5	15	80.6	86.2	74.1	0.30	0.154	18.5	3.7	0.00	81.0	77.8
5	5	16	81.5	86.7	77.5	0.31	0.171	17.2	4.5	0.00	81.0	78.3
5	5	17	80.8	86.6	75.3	0.26	0.151	18.0	4.2	0.00	81.1	77.9

5	18	80.9	86.4	77.2	0.23	0.139	18.5	4.7	0.00	79.8	77.6
5	19	81.0	86.4	76.2	0.26	0.142	17.0	4.0	0.00	80.1	77.8
5	20	81.9	86.9	78.9	0.27	0.147	20.2	5.1	0.07	80.4	78.3
5	21	80.9	86.3	74.8	0.27	0.150	17.5	4.2	0.00	80.8	77.9
5	22	80.6	87.2	75.3	0.22	0.133	19.0	4.5	0.00	80.2	77.4
5	23	81.5	86.6	78.1	0.21	0.130	21.7	6.2	0.00	79.0	77.4
5	24	81.7	86.7	77.7	0.27	0.156	24.5	7.6	0.20	79.3	77.5
5	25	82.8	87.0	79.3	0.31	0.174	20.2	8.4	0.00	79.9	77.7
5	26	81.9	87.8	78.1	0.23	0.142	19.0	5.9	0.00	80.2	77.8
5	27	82.1	87.0	78.2	0.21	0.126	16.3	6.4	0.00	79.0	77.4
5	28	82.1	87.1	77.1	0.27	0.153	16.7	4.3	0.00	79.4	77.4
5	29	80.7	87.6	73.8	0.28	0.156	16.2	3.9	0.00	79.8	77.0
5	30	80.2	87.0	73.3	0.21	0.119	15.7	2.9	0.00	79.5	76.8
5	31	78.3	85.9	70.0	0.24	0.125	13.3	2.0	0.11	78.3	75.5
6	1	79.7	86.4	72.7	0.28	0.152	15.5	2.5	0.02	78.5	75.7

† Indicate average air temperature

‡ Indicate maximum air temperature

§ Indicate minimum air temperature

¶ Indicate average solar radiation

# Indicate potential evaporation

†† Indicate maximum wind speed

‡‡ Indicate average wind speed

§§ Indicate total rainfall

¶¶ Indicate maximum soil temperature

## Indicate minimum soil temperature

Appendix B. Overall genotypic ranking determined by genotypic ranking from 1999 and 2000 yield data, total root length (TRL) from the abscisic acid (ABA) and control growth pouch and polyvinyl chloride (PVC) studies, 1999 and 2000 geometric mean (GM), and 1999 and 2000 N fixation using the non-nodulating DOR 364 as the reference crop. 1 = highest and 8 = lowest.

Genotypes	1999Y	2000Y	ABA TRL	Cont. TRL	PVC TRL	1999 GM	2000 GM	1999 N	2000 N	Rank
BAT 477 (nod)	1036 (5)	804 (7)	39.3 (8)	34.2 (4)	170 (5)	1022 (5)	780 (5)	4.15 (4)	4.9 (2)	5
PR9603-22	1476 (2)	838 (3)	43.0 (6)	33.2 (5)	163 (6)	1439 (2)	831 (2)	3.96 (5)	0.3 (7)	3
DOR 364 (nn)	1256 (4)	896 (1)	76.0 (2)	32.2 (6)	119 (8)	1254 (4)	879 (1)	— (8)	— (8)	4
XAN 176	1397 (3)	865 (2)	60.4 (4)	43.0 (3)	195 (1)	1382 (3)	754 (7)	15.4 (2)	1.4 (4)	2
BAT 477 (nn)	756 (6)	815 (5)	49.4 (5)	45.3 (2)	193 (2)	755 (6)	774 (6)	— (8)	— (8)	7
SEAS	633 (8)	810 (6)	64.6 (3)	31.1 (7)	191 (3)	632 (8)	807 (3)	12.1 (3)	1.0 (5)	6
8-42-M-2	739 (7)	568 (8)	88.2 (1)	26.0 (8)	191 (3)	653 (7)	548 (8)	-6.7 (6)	2.2 (3)	8
DOR 364 (nod)	1508 (1)	825 (4)	41.3 (7)	55.8 (1)	135 (7)	1508 (1)	788 (4)	19.7 (1)	5.3 (1)	1





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